Effects of Small Intravenous Doses of Epinephrine on Serum Insulin, Glucose Tolerance and Serum Free Fatty Acids

P. V. Cardon, M.D., J. L. Weiss, M.D., and P. S. Mueller, M.D., Bethesda

SUMMARY

Eighteen intravenous glucose tolerance tests (IVGTT) were carried out in six young, normal subjects while they were receiving normal saline or epinephrine (E) intravenously at the low rates of 0.1 and 0.3 μgm. per minute. The lower dose of E caused changes in the rate of urinary excretion of free E similar to those caused by caffeine; the higher dose caused changes similar to those reported to occur during various moderate to severe stresses. The higher dose of E augmented the insulin response to IVGTT. Glucose tolerance was not significantly decreased, resulting in relatively low glucose-insulin ratios following glucose injection. Serum free fatty acids were unaffected. DIABETES *23*:743-47, September, 1974.

It is well known that epinephrine (E) affects carbohydrate and fat metabolism in man, and that the rate of E secretion may increase in stressful or frightening circumstances. There is little information with which to relate these phenomena to each other in a quantitative manner. In the present study, E was infused intravenously at rates calculated to increase urinary excretion of free E by amounts similar to the increases often seen clinically in a variety of stressful circumstances. These small doses of E had no detectable effect on serum free fatty acids (FFA), and their inconstant effects on glucose tolerance were not statistically significant. However, the larger dose resulted in significantly elevated serum insulin levels after intravenous glucose administration.

METHODS

Six normal volunteers, four women and two men aged eighteen to twenty years, were studied. They were in good health; none had a family history of diabetes. Fasting and postprandial blood glucose levels were within normal limits. For at least three days before and during the period of study, their daily diets contained at least 300 gm. carbohydrate.

Each subject was studied three times on separate mornings. Each received three treatments, one subject being assigned by lot to each of the six possible treatment sequences. The three treatments were: Intravenous normal saline solution only (TS); E 0.1 μgm./min. (TE .1); E 0.3 μgm./min. (TE .3). 1:1000 solutions of 1-E were freshly diluted in 1 L. of normal saline. Ascorbic acid (2 mg.) was added to all treatment solutions as an antioxidant. Volume rate of infusion was controlled by a peristaltic action pump and was the same for the three treatments (2.5 ml. per minute). The subjects were "blind" to treatment sequences.

On the morning of each study, the fasting subject voided at zero time, about 9 a.m., and then lay quietly in bed while an intravenous infusion of normal saline was started in the left arm and a venous blood sampling catheter was placed in the right arm. At thirty minutes, a ninety-minute "treatment period" was begun by turning on the infusion pump and turning a stopcock located near the infusion needle. At fifty minutes, 25 gm. of glucose in 50 per cent solution was given through a Y tube into the left arm vein over a three-minute period without interrupting the pump flow. Eight 10-ml. blood samples were taken from the right arm at 25, 45, 55, 65, 75, 85, 100 and 115 minutes (five minutes before and fifteen minutes after starting treatment and 5, 15, 25, 35, 50 and 65 minutes after start of glucose injection). Samples were immediately placed in ice. At 120 minutes the needles were removed and the subject voided a two-hour urine sample which was immediately frozen.

The chilled clotted blood samples were centrifuged; the serum was removed and immediately frozen. Serum samples were identified by code numbers so

From the Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, Maryland, and the Department of Psychiatry, Yale University.

Address reprint requests to: P.V. Cardon, M.D., National Institute of Mental Health, 9000 Rockville Pike, Bethesda, Maryland 20014.

Accepted for publication May 27, 1974.

that chemical analyses were done without knowledge of the subject, treatment, or time of collection. Urine samples were analyzed without knowledge of the treatments.

Serum glucose was determined by a modification of the glucose oxidase method.[1] FFA was measured titrimetrically by the method of Mueller and Watkin.[2] Insulin was determined by double antibody radioimmunoassay.[3] Urinary free E and norepinephrine (NE) were determined fluorimetrically as described elsewhere.[4]

The fractional rate of glucose disappearance (K) was computed from the glucose concentrations in the four samples drawn 15, 25, 35, and 50 minutes after the start of glucose injection. The slope of the log-transformed values was computed by the method of least squares. The five- and sixty-five-minute samples were not used for this computation because the former time may be too soon for uniform distribution of the injected glucose, and by sixty-five minutes the values are not infrequently at or below the pre-injection level.[5,6]

The excess urinary E (EX) attributed to the administration of E was computed from the formula

$$EX = E_E - E_S$$

where E is the quantity of free E in a two-hour urine sample and the subscripts E and S denote E-infusion day and saline-only day, respectively. The rate per minute of excretion of excess urinary E (REX) is:

$$REX = EX/90$$

The total rate per minute of E excretion (excess E plus endogenous E) during the ninety-minute treatment period (RET) is:

$$RET = REX + E_S/120$$

Statistical significance of treatment effects was assessed by two-way analyses of variance,[7] taking treatments and subjects as main effects (for treatments, df are 2,10).

RESULTS

All subjects stated that they experienced none of the familiar bodily sensations associated with fear, nor those caused by larger dose of E, such as forceful heart beat, tremor, and cold extremities. None could distinguish between E infusion and saline infusion.

The average values for the variables measured will be given in the following order: Saline-only day (TS), lower dose of E day (TE .1), and higher dose of E day (TE .3).

Urinary epinephrine and norepinephrine. Without exception, the amount of free E in the urine was lowest on the TS day and highest on the TE .3 day ($F = 17.29$, $p < .001$). Average quantities excreted (μgm./2 hr.) were: .36, .99 and 1.56. Average computed rates of E excretion during the ninety-minute treatment periods (RET, ng/min.) were: 3.0, 10.0 and 16.4. There was no treatment effect on NE excretion. Average values (μgm./2 hr.) were: 1.9, 2.0 and 2.0 ($F = .05$, $p > .95$).

Serum Insulin. Results are summarized in table 1. Average serum insulin concentrations in the first and second blood samples were similar on the three days and there were no differences among the average changes from first to second sample ($F = .02$, $p > .95$). In other words, E infusion by itself had no effect on serum insulin concentrations.

In the samples taken five minutes after starting glucose injection, average insulin concentrations were similar and the differences do not approach statistical significance ($F = .36$). At the five subsequent sampling times (fifteen to sixty-five minutes after glucose injection), average values were 12-37 μU greater during TE .3 than during TS, and the average values for TE .1 usually were intermediate. Probabilities of chance occurrence of the observed average differences at the last five sampling times are less than .1, .1, .05, .1, and .01, respectively. Computing the areas under the post-glucose insulin curves, average values (μU x min.) were: 3275, 3500 and 4062 (F 7.50, $p = .01$). Areas of the post-glucose insulin increments, (baseline defined as average of first two samples), were 2542, 2600 and 3242 (F 6.55, $p < .02$).

Serum Glucose. There were no statistically significant treatment effects on glucose concentration or on the computed fractional rate of disappearance (K). The statistically nonsignificant differences among averages were, however, in the predicted order and direction for the following parameters: Change from first to second sample ($F = .87$), concentrations in the last five samples, areas under the post-injection curves, and K (table 1). (The predictions, from known effects of large doses of E,[8] were that there would be dose-related increases in glucose and decreases in K).

Glucose/Insulin Ratio. Following glucose injection, glucose/insulin ratios tended to be smaller during TE than during TS. The last blood sample was the only time point at which the differences among treatments were statistically significant ($F = 4.31$, $p < .05$). The differences among the averages of glucose-area/insulin-area ratios approach statistical significance ($F = 3.76$, $< .06$).

TABLE 1

Effects of epinephrine infusion on serum insulin, intravenous glucose tolerance and free fatty acids.

Sample	Time	25′ (T)	45′ (G)	55′	65′	75′	85′	100′	115′	Area 55′-115′ (Units x min.)	K (%/min.)
Insulin	TS	13.2	11.8	125.0	69.3	53.0	47.8	36.5	27.7	3,275	
(μU)	TE.1	15.8	14.5	117.7	74.7	52.3	49.7	49.0	37.8	3,500	
	TE.3	13.8	13.0	118.9	82.2	65.0	68.5	50.5	50.8	4,062	
	F	.70	.39	.36	4.05	3.33	4.77	3.96	10.00	7.50	
Glucose	TS	88.2	86.7	229.0	206.0	174.2	149.0	124.5	112.0	9,517	1.50
(mg. %)	TE.1	85.3	85.5	250.0	217.7	187.5	164.3	143.2	125.0	10,443	1.19
	TE.3	83.8	86.3	236.0	219.2	189.7	171.2	146.2	126.8	10,558	1.15
	F	1.34	.13	1.35	2.77	2.59	1.96	1.80	1.53	2.11	1.26
Glucose*	TS	7.5	8.5	2.1	3.3	3.9	3.9	4.1	5.1	3.29	
Insulin	TE.1	6.4	6.4	2.3	3.2	4.1	3.8	3.1	4.0	3.24	
(mg. %/μU)	TE.3	8.4	9.9	2.1	3.1	3.2	2.7	3.2	2.7	2.75	
	F	.95	2.14	.33	.36	2.35	2.95	2.10	4.31	3.75	
FFA	TS	.36	.33	.35	.28	.23	.21	.16	.18	13.3	
(mEq/L)	TE.1	.24	.42	.33	.26	.23	.19	.19	.21	13.4	
	TE.3	.24	.33	.31	.30	.22	.20	.18	.25	14.0	
	F	3.45	1.30	.11	.32	.03	.07	.16	1.02	.17	
For df 2,10:		P	.95	.2	.1	.05	.01				
		F	.05	1.90	2.92	4.10	7.56				

Explanation of symbols: (T), 90-min. treatment period began at 30′; (G), 3-min. injections of 25 gm. glucose began at 50′; TS, saline treatment only; TE.1, epinephrine 0.1 μgm./min. IV; TE.3, ditto 0.3 μgm./min.
*Averages of 6 ratios, therefore different from ratios of tabulated glucose and insulin averages.

TABLE 2

Reported effects of various stresses on epinephrine excretion.

Reference	Assay Method*	Acid Hydrolysis**	Type of Stress	Urinary E ng./min. Means (high range) Control	Stress	Remarks
9	Bio.	no?	Childbirth	3.7(7.7)	9.5(61)	24 hr.
10	Bio.	yes	Hockey, Boxing, LSD reaction	5.3 3.5	14 (27) 8 (58)	High range computed from S.E.M.
11	Bio.	yes	Thermal burns	11	47 (102) 73 (180)	Patients recovered Patients died Averages for first days
12	Bio.	no	Major Surgery	3.1(10.8)	8.0(58)	Stress mean is for first 3 post-operative days. High range 1 patient on 1 day
13	Chem.	yes?	Anxiety with Depression	12.7	16.7	24 hr.
14	Chem.	no	Industrial stress	10.7(17.5)	17.5(54)	High range computed from SD
15	Chem.	no	Hospital admission	4.8	7.3(13)	24 hr.
16	Chem.	no	Myocardial infarction	2.6	(9)	24 hr.
17	Chem.	yes	Psychotic depression	(11)	11 (14)	Stress values are means of 16 24-hr. samples in each patient. Some single days were 8 times normal.
This study	Chem.	no	E infusion 300 ng./min.	3 (6)	16.4(29)	

*Early studies employed bioassay, later studies chemical methods.
**When acid hydrolysis was done, total epinephrine excretion rates (free plus conjugated) were reported.

Serum Free Fatty Acids. There were no significant differences among treatments at any time. Compared to insulin and glucose, the average differences and F ratios are much smaller. The average differences are not consistently in the order or direction predicted from the known lipolytic effects of larger doses of E.

DISCUSSION

There have been no studies in humans of glucose tolerance or of insulin response to glucose infusion, in relation to some index of normal adrenal medullary function. There are, however, many reports of the effects of a variety of stresses on the rate of urinary excretion of E (9-17). Some of those data are summarized in table 2. Excretion rates originally reported as rate/24 hr. or as rate/unit creatinine have been converted for tabulation to ng/min.

The infusion of E at a rate of 0.3 μgm./min. resulted in an average rate of urinary excretion which is similar to most of the average rates during stress shown in the table. Some individuals, particularly during severe stress such as psychotic depression, childbirth, major surgery, and especially burns, had excretion rates several times higher than the highest rate seen in the present study. We conclude that 0.3 μgm./min. is a fair approximation of the increments in endogenous secretion rate which usually occur during stress in humans, that much larger increments do occur, but are probably relatively infrequent. Infusion of 0.1 μg/min. resulted in increments in E excretion similar to reported effects of coffee ingestion.[18,19]

One may ask whether such small doses of E are indeed effective or "physiological." The evidence summarized above indicates at least that the resulting rates of E excretion are similar to those which usually occur naturally. Also, the small doses of E used in the present study have small but statistically significant effects on circulatory function.[20]

In the present study, the only consistent effect was potentiation of the later phase of the insulin response to intravenous glucose, during infusion of E at a rate of 0.3 μgm./min. The glucose data are inconclusive. One can neither say with confidence that effects were observed, nor that the differences observed should be ascribed to chance. Despite this ambiguity, the data favor the practical conclusion that in humans the amounts of E usually secreted under stressful circumstances impair glucose tolerance only slightly, if at all.

In contrast to insulin and glucose, FFA clearly were not affected by E infusion. This indicates that the rapid increases in FFA, which consistently occur under moderately stressful circumstances,[21,22] are not the result of increases in circulating E. An alternative mediating mechanism is increased activity of sympathetic nerves of adipose tissue.[23]

Except for the increased insulin response, the data do not support the possibility that increased secretion of E is responsible for the abnormalities observed in psychotic depressed patients,[24,25] namely elevated fasting glucose and FFA, decreased K and increased insulin response to IVGTT. Similarly, it appears that the increased FFA and decreased glucose tolerance with high glucose/insulin ratios, caused by caffeine,[26,27] cannot be ascribed to augmented secretion of E.

Returning to the apparent potentiation of the insulin response to glucose infusion: There is no other clinical evidence that E can enhance insulin secretion in the absence of alpha adrenergic blockage. In the most pertinent previous clinical study, a stimulating effect of E on insulin secretion was observed only after administration of an alpha blocker.[28] In the absence of alpha adrenergic blockade, E has heretofore been reported to inhibit the insulin response to glucose infusion.[29,30] However, the doses of E employed in the above studies were 5 to 6 μgm./min., roughly twenty times the higher dose used in the present study.

Pretreatment with the beta blocker propanolol diminishes the insulin response to glucose infusion in humans, but does not affect fasting serum insulin levels.[31] The parallel to the present findings is apparent; fasting insulin was unaffected by E, but the response to glucose infusion was potentiated. Propanolol does, however, decrease fasting insulin in baboons.[32]

It is postulated that in very low concentrations E enhances insulin response to glucose challenge by a mechanism susceptible to beta blockade, while in higher concentrations it inhibits insulin secretion by a mechanism susceptible to alpha blockade.

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