Prostaglandin E_2 Metabolite Levels During Diabetic Ketoacidosis

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SUMMARY

Insulin therapy was withdrawn from 15 well-controlled type I diabetic subjects for no longer than 18 h to examine the sequence with which 13,14-dihydro-15-keto-PGE_2 (PGE-m), glucagon, norepinephrine, and epinephrine increased in circulating blood in diabetic subjects becoming ketoacidotic. Fourteen of 15 patients had increments in PGE-m; 12/12, 12/15, and 13/15 had increments in glucagon, norepinephrine, and epinephrine, respectively. Six of the 15 patients developed mild diabetic ketoacidosis (DKA) by 12–18 h; all had nonmeasurable C-peptide levels. This DKA group had significantly greater increments of PGE-m (835 ± 130 versus 276 ± 111 pg/ml, mean ± SEM, $P < 0.01$) but not glucagon, norepinephrine, or epinephrine compared with the 9 non-DKA patients. In the DKA group, there were significant PGE-m and glucagon increments in the circulation by 3 h, significant norepinephrine increments by 9 h, and epinephrine increments in 5/6 patients by 12 h (not statistically significant) of insulin withdrawal. These studies document that (1) PGE-m accumulates in the circulation during DKA, (2) PGE-m and glucagon increase before catecholamines, and (3) PGE-m, glucagon, and catecholamine levels promptly return to normal levels when insulin therapy is reinstituted. It is suggested that elevated PGE-m levels early in the onset of DKA may represent a host-defense mechanism.

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Diabetic ketoacidosis (DKA) is characteristically associated with hyperglycemia, insulin deficiency, increased circulating levels of fat-derived fuels, such as free fatty acids and ketone bodies, as well as increased levels of lipolytic hormones, such as glucagon and catecholamines. Axelrod et al. reported increased circulating levels of 15-keto-13,14-dihydro-prostaglandin E_2 (PGE-m), a major circulating metabolite of PGE_2, in rats rendered ketoacidotic by treatment with streptozocin.[1] The latter findings have potential relevance to the pathophysiology of DKA in humans, because PGE_2 binds to human fat cells,[2–8] inhibits fat cell adenylate cyclase[9] and lipolysis,[10–15] and modulates secretion of catecholamines[16] and pancreatic islet hormones.[17]

The investigation reported herein used a brief period of insulin withdrawal in 15 type I diabetic subjects and plasma measurements to determine whether PGE-m levels increase in humans during DKA; to characterize temporally the PGE-m responses to the responses of glucagon, norepinephrine, and epinephrine; and to characterize the behavior of all these substances after reinstitution of insulin therapy. Measurements were also made of circulating glucose, C-peptide, free fatty acids, glycerol, beta-hydroxybutyrate, and pH.

MATERIALS AND METHODS

Study protocol. Fifteen type I, insulin-treated, fully informed, and fully consenting diabetic subjects (9 men, 6 women, 31 ± 8 yr old, mean ± SD) with a history of spontaneous ketoacidosis were studied. This study was approved by the University Human Subjects Committee and the Clinical Research Center Scientific Advisory Board. Three days before admission, the patients' usual regimen of intermediate-acting insulin was discontinued. Subcutaneous injections of regular insulin were then given for 3 days using 150% of the usual daily dose of long-acting insulin. The daily amount was divided into four doses (2/7, 2/7, 2/7, and 1/7) given 30 min before three meals and a bedtime snack. Ingested calories were divided in a similar fashion throughout the day, i.e., 2/7, 2/7, 2/7, and 1/7. On the fourth day, the patients were admitted to the Clinical Research Center in the morning for a history, physical examination, and electrocardiogram. At 4:30 p.m. on this day, a 19-gauge needle was placed in an antecubital vein and kept patent with a slow infusion of saline

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TABLE 1
Basal, maximal, and final circulating concentrations of fuels and [H^+] during insulin withdrawal in 15 type I diabetic subjects

		Glucose (mg/dl)			Free fatty acids (μeq/L)			Glycerol (μmol/ml)			β-OH-B (μmol/ml)			[H^+] (pH)		
Patient	(h)	Basal	Max	Final	Basal	Max	Final	Basal	Max	Final	Basal	Max	Final	Basal	Max	Final
1	(12)	353	786	786	968	2156	2156	0.047	0.180	0.180	0.925	3.82	3.82	7.37	7.33	7.33
2	(12)	201	434	368	843	3297	3297	0.068	0.278	0.278	0.594	3.25	3.25	7.37	7.34	7.34
3	(15)	236	656	632	755	1555	1465	0.112	0.194	0.166	0.249	8.21	8.13	7.37	7.20	7.20
4	(15)	236	828	828	520	2514	2514	0.051	0.332	0.332	0.187	5.04	5.04	7.41	7.31	7.31
5	(18)	68	705	460	426	1523	1523	0.029	0.136	0.136	0.059	5.11	5.11	7.45	7.34	7.34
6	(18)	139	758	656	154	3290	3149	0.009	0.337	0.337	0.042	4.50	4.50	7.37	7.28	7.28
7	(18)	206	600	504	232	1642	1371	0.016	0.095	0.089	0.090	3.08	3.08	7.38	7.36	7.37
8	(18)	262	696	632	561	1304	1075	0.044	0.116	0.053	0.211	2.82	2.82	7.35	7.37	7.37
9	(18)	162	540	396	481	1140	1097	0.051	0.137	0.108	0.347	1.91	1.91	7.42	7.40	7.40
10	(18)	91	425	337	890	1272	1272	0.081	0.113	0.113	0.908	2.35	1.86	7.37	7.37	7.37
11	(18)	162	542	462	570	1209	1075	0.034	0.061	0.061	0.169	1.05	1.05	7.41	7.38	7.40
12	(18)	309	592	338	405	715	715	0.038	0.072	0.072	0.141	1.01	1.01	7.40	7.39	7.39
13	(18)	155	376	311	861	803	672	0.071	0.071	0.060	0.363	0.327	0.347	7.41	7.41	7.45
14	(15)	111	298	212	842	1242	991	0.097	0.096	0.084	0.170	0.414	0.455	7.40	7.41	7.41
15	(18)	129	380	290	389	602	447	0.032	0.050	0.050	0.097	0.145	0.084	7.39	7.38	7.38

The number of hours in parentheses refers to the duration of insulin withdrawal. "Basal" refers to values before insulin withdrawal, while "Max" refers to peak values during withdrawal and "Final" refers to the last value just before insulin treatment was reinstated. Patients 1 through 6 developed mild ketoacidosis.

(0.9% sodium chloride). An i.v. infusion of regular insulin in saline by a Harvard perfusion pump was begun at the initial rate of 0.5 U/h. In addition, 5 U of regular insulin was given i.v. 30 min before supper. Blood glucose levels were measured at 9 p.m. and midnight so that the insulin infusion could be adjusted if necessary to avoid extremes in circulating glucose levels.

At 6 a.m. the following morning, the patients were weighed and blood samples were obtained. At 6:30 a.m. the i.v. infusion of insulin was discontinued for a period of time no longer than 18 h. The diet and oral fluids were continued as desired by the patients. The patients' weight, fluid intake and output, heart rate, supine and sitting blood pressure, and mental status were continuously monitored. Blood samples were obtained every 3 h. The criteria used for attainment of diabetic ketoacidosis were: (1) plasma glucose >450 mg/dl or an increase in glucose >150 mg/dl, (2) plasma ketones >1:4 dilution, and (3) arterialized venous blood CO_2 content <14 meq/L or decrease in CO_2 content >12 meq/L or pH <7.25.

Once these criteria were met, diabetic ketoacidosis was reversed by giving the patients one regular insulin injection (5 U i.v.) and an insulin infusion (3 U/h i.v. in normal saline) until the plasma glucose was <250 mg/dl, blood CO_2 content was >22 meq/L, and blood pH was >7.35. Fluid therapy during this time consisted of 1 L of normal saline over 1 h, then 500 cc/h for 2 h, then 250 cc/h until the plasma glucose was <250 mg/dl. Fluid therapy was then changed to 5% dextrose and 0.45% saline. Potassium chloride (20 meq/L) was added to the infusion bottles when the serum potassium was <4.5 meq/L. Fluids were discontinued when the criteria given above for discontinuing i.v. insulin were met. Blood samples were continued every 3 h during i.v. insulin infusion. After achieving control of ketoacidosis, the regimen of s.c. injections of regular insulin ½ h before meals four times daily described above was used for 1 day and then the usual dose of long-acting insulin was reinstituted.

Measurements. Glucose,[18] C-peptide,[19] free fatty acids,[20] glycerol,[21] beta-hydroxybutyrate,[22] norepinephrine,[23] epinephrine,[23] and glucagon[24] were quantified by standard

TABLE 2
Basal, maximal, and final circulating concentrations of PGE-m and hormones during insulin withdrawal in 15 type I diabetic subjects

		PGE-m (pg/ml)			Glucagon (pg/ml)			Norepinephrine (pg/ml)			Epinephrine (pg/ml)			C-peptide (pg/ml)	
Patient	(h)	Basal	Max	Final	Basal	Max	Final	Basal	Max	Final	Basal	Max	Final	Basal	Final
1	(12)	550	2000	1880	49	86	81	90	430	430	35	70	70	<200	<200
2	(12)	610	1560	1200	56	165	165	195	440	440	40	100	100	<200	<200
3	(15)	320	820	540	53	85	80	105	420	420	110	180	140	<200	<200
4	(15)	230	940	900	62	145	145	180	430	430	40	70	70	<200	<200
5	(18)	<20	840	820	—	—	—	150	170	170	115	50	40	<200	<200
6	(18)	118	1000	640	27	143	104	105	240	220	45	70	50	<200	<200
7	(18)	63	230	230	—	—	—	70	70	50	55	130	70	—	—
8	(18)	390	1480	1200	78	161	161	75	210	170	50	90	80	<200	<200
9	(18)	173	130	<50	—	—	—	105	220	120	35	80	30	204	208
10	(18)	80	250	112	32	71	71	255	280	280	70	110	110	<200	<200
11	(18)	110	240	160	63	85	60	205	110	160	50	90	90	—	—
12	(18)	190	460	420	138	185	170	115	120	90	55	50	40	—	—
13	(18)	236	680	270	135	175	165	200	260	190	70	140	100	816	1022
14	(15)	168	340	200	82	97	87	485	400	440	60	80	40	1477	1201
15	(18)	<20	100	70	52	77	66	180	250	180	35	50	20	558	1261

See legend to Table 1 for explanation of terms.

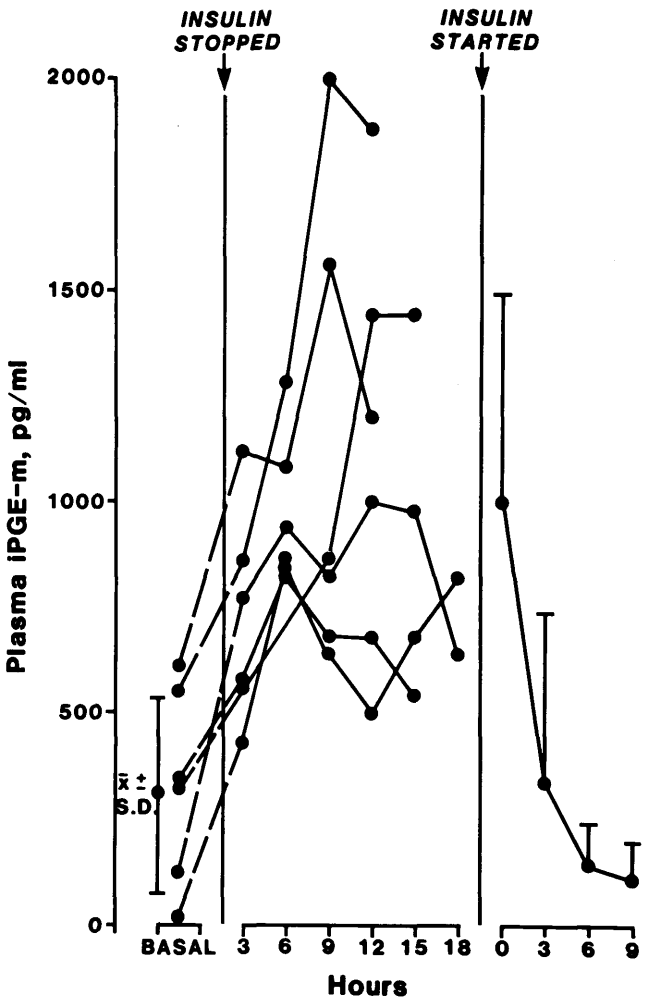

FIGURE 1. Plasma PGE-m levels before, during, and after withdrawal of i.v. insulin infusions in the 6 of 15 type I diabetic subjects who developed mild DKA in 12–18 h. Reinstatement of insulin therapy promptly returned PGE-m values (mean ± SD) to pre-experimental levels.

methods. PGE-m was measured using unextracted plasma and the identical antiserum directed against 15-keto-13,14-dihydro-PGE_2[25] that was used by Axelrod et al.[1] and was generously donated by Dr. L. Levine.

Statistics. Nonpaired testing was performed by Student's *t*-test to compare data from the group of patients who developed DKA with the group that did not.

RESULTS

Patient responses. Basal levels of the circulating fuels, PGE-m, and hormones were calculated by averaging the two values obtained from blood samples drawn 30 min and again immediately before discontinuance of the insulin infusions (Table 1). The maximal values represent the peak levels observed during the period of insulin withdrawal. The final values were obtained from samples drawn just before insulin therapy was reinstated. Of the 15 patients studied, circulating glucose and free fatty acids and/or glycerol were increased in all and beta-hydroxybutyrate rose in 14. Six of the 15 patients developed DKA according to our prospective criteria within 18 h of insulin withdrawal. There were significantly greater (mean ± SEM) maximal levels of glucose (695 ± 58 versus 517 ± 55 mg/dl, $P < 0.05$), free fatty acids (2389 ± 324 versus 1103 ± 111 μeq/L, $P < 0.001$), glycerol (0.243 ± 0.036 versus 0.090 ± 0.010 μmol/ml, $P < 0.001$), beta-hydroxybutyrate (4.99 ± 0.706 versus 1.49 ± 0.361 μmol/ml, $P < 0.001$), and $[H^+]$ (7.30 ± 0.02 versus 7.38 ± 0.01, pH, $P < 0.001$) in the DKA group (6 patients) compared with the non-DKA group (9 patients). Circulating levels of C-peptide were measured in samples from 12 randomly selected patients. None of the six patients in the DKA group had measurable levels of C-peptide, but four of the six patients in the non-DKA group did (Table 2). Fourteen of the 15 patients had increases in PGE-m (Table 2). The magnitude of the changes in PGE-m (maximal less basal level) in the DKA group was greater than in the non-DKA group (835 ± 130 versus 276 ± 111 pg/ml, mean ± SEM, $P < 0.01$). Plasma levels of glucagon, norepinephrine, and epinephrine rose in 12/12, 12/15, and 13/15 patients, respectively (Table 2). No significant differences in these hormone responses were observed between the DKA and non-DKA groups.

Time course of changes in PGE-m and hormones. The temporal sequence with which changes were observed in circulating PGE-m, glucagon, norepinephrine, and epinephrine was examined by plotting the individual data (Figures

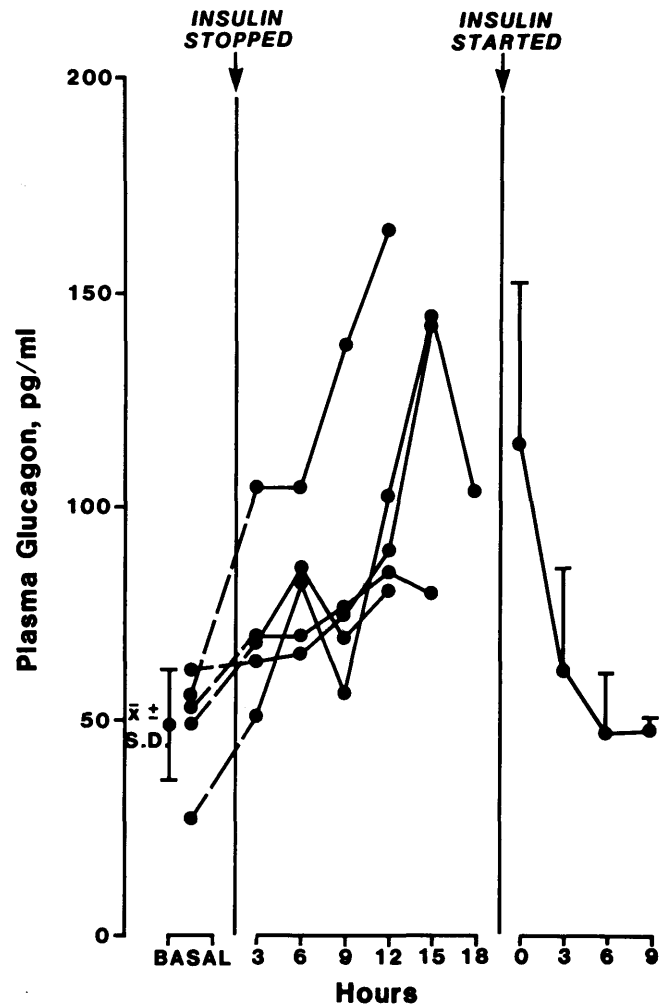

FIGURE 2. Plasma glucagon levels in 5 of the 6 DKA patients (1 set of samples was lost) presented in Figure 1 (mean ± SD).

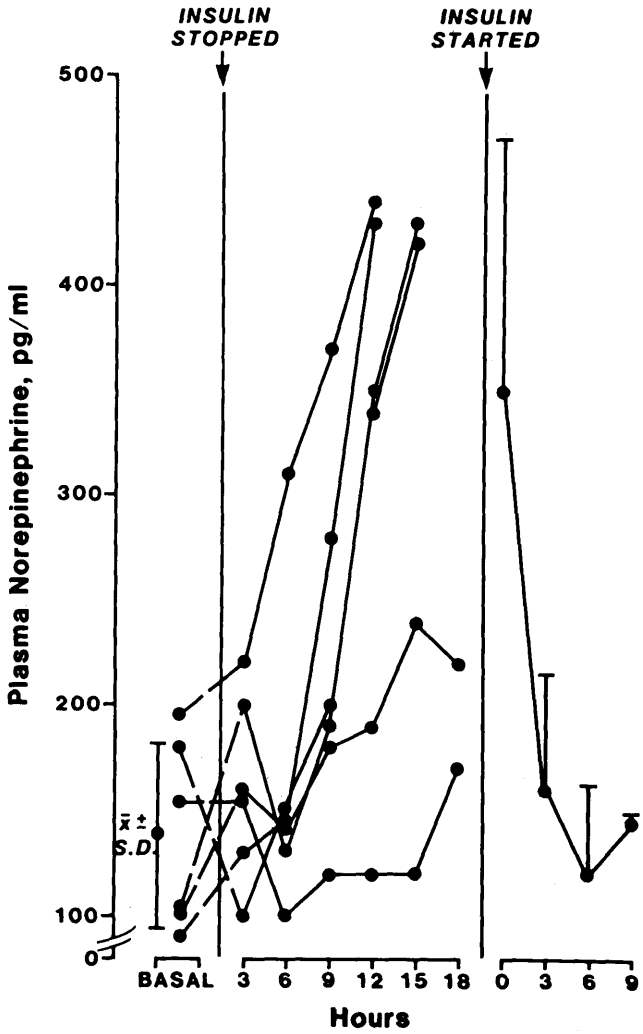

FIGURE 3. Plasma norepinephrine levels in the 6 DKA patients presented in Figure 1 (mean ± SD).

1–4) and mean data (Figure 5) for the six patients who became ketoacidotic. Plasma PGE-m levels promptly increased in all six patients by 3 h and thereafter continued to rise (Figures 1 and 5). At 3 h, the increase (mean ± SEM) over basal for PGE-m was 373 ± 78 pg/ml. On reinstatement of insulin therapy, the PGE-m increases promptly fell and by 6 h were within the basal range observed before insulin withdrawal. Plasma glucagon levels were increased in 5/5 patients by 3 h after insulin withdrawal and thereafter steadily increased (Figures 2 and 5). These elevated levels fell promptly after insulin therapy and by 6 h postinfusion were within the basal range observed before insulin was withdrawn. There was greater variability in the plasma norepinephrine responses (Figures 3 and 5). Consistent increases in circulating norepinephrine were not observed until 9 h after insulin withdrawal. These levels promptly fell after insulin therapy and were within the basal range by 6 h. No consistent trend was observed in the plasma epinephrine responses until the twelfth hour, when 5/6 patients had increases over basal levels (Figures 4 and 5). However, in most patients this response was not as sustained as the norepinephrine responses and, consequently, reinstatement of insulin therapy did not cause a dramatic decrease in epinephrine as was observed with PGE-m, glucagon, and norepinephrine.

DISCUSSION

Axelrod et al.[1] published that rats made diabetic and ketoacidotic by treatment with streptozocin develop elevated circulating levels of 15-keto-13,14-dihydro PGE_2. These authors verified their measurements using high-performance liquid chromatography before radioimmunoassay of this metabolite. Our own experience[25] indicates that this antiserum weakly recognizes (cross-reactivity of 15%) free fatty acids. When the appropriate calculations are performed using the measured increases in free fatty acids, such cross-reactivity is not sufficient to account for the magnitude of PGE-m levels in humans that we report. This study was designed to determine whether PGE-m levels in type I diabetic subjects increase during the onset of DKA and to examine the temporal relationship between changes in PGE-m, glucagon, norepinephrine, and epinephrine. All of the 15 patients had increases in circulating glucose, fat-derived fuels, glucagon,

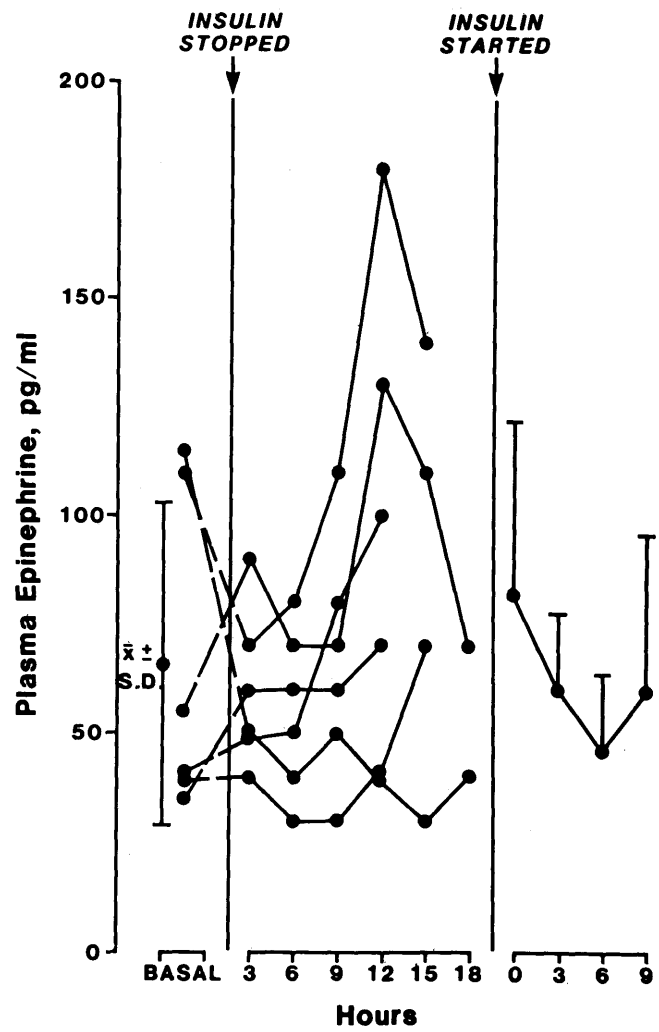

FIGURE 4. Plasma epinephrine levels in the 6 DKA patients represented in Figure 1 (mean ± SD).

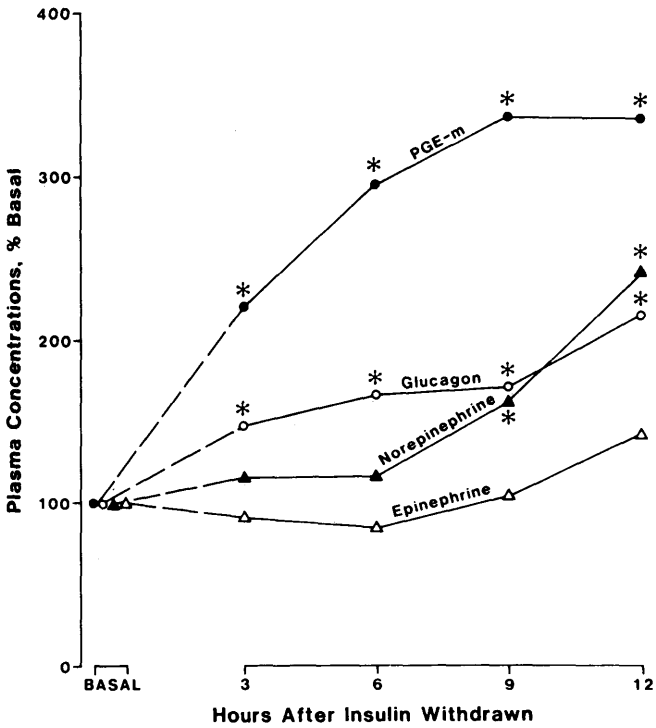

FIGURE 5. Mean plasma values of the PGE-m, glucagon, norepinephrine, and epinephrine levels shown in Figures 1–4. There were statistically significant (*P < 0.05) increments in PGE-m and glucagon by 3 h, in norepinephrine by 9 h, but not in epinephrine by 12 h.

and either norepinephrine or epinephrine during insulin withdrawal. Six of these subjects became mildly ketoacidotic. PGE-m was increased in 14/15 subjects; significantly greater levels were observed in the six patients who became ketoacidotic compared with the nine who did not. In the six DKA patients, PGE-m and glucagon appeared in the circulation by 3 h and before there were increases in catecholamines. Levels of norepinephrine were not convincingly elevated until 9 h of insulin withdrawal had elapsed, and epinephrine levels were only questionably elevated. Elevated levels of PGE-m and the three hormones promptly decreased after reinstatement of insulin therapy.

The potential physiologic relevance of the data previously published by Axelrod et al.[1] and the data in this article to the syndrome of diabetic ketoacidosis depends heavily on previous observations concerning adipocyte PGE_2 receptors,[2–8] PGE_2-inhibition of adenylate cyclase activity,[9] the antilipolytic effect of PGE_2,[10–15] and synthesis of PGE_2 by the adipocyte.[26–28] For example, it is known that PGE_2 binding sites on human adipocytes exist and that these sites have a dissociation constant that is comparable to the IC_{50} for the antilipolytic effect of PGE_2.[7,8] Although it has been appreciated for some time that PGE_2 has effects on cyclic AMP generation by adipocytes and other cells, Kather et al.[9] recently described a biphasic effect of PGE_2 on human fat cell adenylate cyclase activity. Inhibition occurred at nanomolar concentrations and stimulation occurred at concentrations greater than 10^{-6} molar. It is generally assumed that inhibition of adenylate cyclase activity is the mechanism whereby PGE_2 exerts its antilipolytic effect.[10–15] Since PGE_2 is not a hormone and in normal situations is not found in the general circulation, the observation that the fat cell itself synthesizes PGE_2[27,28] is particularly important to the hypothesis[26,27] that fat cell generation of PGE_2 may represent a local counterregulatory event during lipolysis. Although the source of the PGE_2 metabolite that we measured cannot be determined from our study, it is possible that at least some of this substance was derived from accelerated PGE_2 synthesis by fat cells. This line of reasoning leads to the suggestion that accumulation of PGE-m in the circulation of type I diabetic subjects during DKA may reflect a host-defense mechanism during accelerated lipolysis. It should be noted, however, that there is controversy about the counterregulatory role of PGE_2 on endogenous lipolysis. Most of the conflicting studies have used nonsteroidal anti-inflammatory drugs, which themselves are problematic since they are not specific inhibitors of PGE_2 synthesis. An alternate but not mutually exclusive site of action for PGE_2 in this setting could be the liver, since PGE_2 has been demonstrated to be synthesized by the liver[29] and has been shown to desensitize liver adenylate cyclase responsiveness to glucagon,[30] a major hormonal force in the pathogenesis of DKA.

In conclusion, the PGE-m data from rats previously published by Axelrod et al.[1] and our PGE-m data from human experiments (using the same PGE-m antiserum used by Axelrod et al.) may be relevant to the syndrome of diabetic ketoacidosis because it is known that PGE_2 (1) binds to human fat cells, (2) inhibits adenylate cyclase, and (3) is antilipolytic. Since it is also known that fat cells synthesize PGE_2, it can be hypothesized that PGE_2 synthesis during accelerated lipolysis and incipient DKA may provide local counterregulation of lipolysis in adipose tissue.

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