Increased Integrated Concentration of Norepinephrine, Epinephrine, Aldosterone, and Growth Hormone in Patients with Uncontrolled Juvenile Diabetes Mellitus

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SUMMARY

The 24-h integrated plasma concentration of glucose (IC-glucose), norepinephrine (IC-NE), epinephrine (IC-E), cortisol (IC-F), growth hormone (IC-GH), aldosterone (IC-ALDO), and plasma renin activity (IC-PRA) were measured in 11 nonobese juvenile-onset nonketotic diabetic patients exhibiting hyperglycemia and glycosuria and 34 matched control subjects using a portable pump, drawing blood at a constant rate through a nonthrombogenic i.v. catheter. The diabetic patients had a noticeable rise of their IC-NE, IC-E, IC-GH, and IC-ALDO. There was no significant difference between the IC-F and IC-PRA of the patients and the control subjects. DIABETES 29:655–658, August 1980.

The role of counterregulatory hormones in the glucose homeostasis of diabetic patients is of obvious interest. Previous studies on the blood levels of catecholamines in diabetes did not reveal a consistent pattern. Christensen[1,2] reported normal plasma levels of norepinephrine (NE) and epinephrine (E) in insulin-dependent diabetic patients whose metabolic status was under control, but high levels of NE and E in patients exhibiting ketoacidosis. After correction of ketoacidosis, the levels of NE and E fell back to normal. Alberti[3] showed that plasma NE and E were normal in diabetic patients after withdrawal of their insulin therapy. Halter et al.[4] and Robertson et al.,[5] on the other hand, reported elevated basal levels of plasma catecholamines in nonketotic noninsulin-dependent diabetic patients. Cryer et al.[6] studied 100 insulin-dependent and nondependent nonketotic diabetic patients and found an increased NE and E in only six of them. Christensen[7] found a relatively small increase in plasma catecholamines of diabetic patients during posture change compared with the increase in pulse rate.

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Numerous authors point out the abnormalities of the renin-angiotensin-aldosterone system in patients with long-standing complicated diabetes mellitus.[8–17] Low plasma renin activity (PRA) was reported in long-standing diabetic patients with minimal or absent kidney and neuropathic changes,[12,13] while others reported normal PRA and aldosterone (ALDO).[18–22] Most workers used either a single, basal, supine, plasma sampling or some manipulation of the renin-aldosterone system like salt restriction or posture change. The plasma levels of NE, E,[23] ALDO, PRA,[24–28] and cortisol (F),[29] fluctuate throughout the day. The concentration of these blood constituents in a single, discrete, blood sample often does not reflect the average 24 h integrated concentration (IC).

The determination of the IC of hormones in plasma, rather than the measurement of the concentration of a discrete blood specimen drawn at a single time, has many distinct advantages when studying substances whose levels fluctuate widely during the day. IC is defined as the concentration of a substance in a pool of blood collected by withdrawal at a constant rate from a peripheral vessel. The result of such a determination is somewhat similar, although more accurate than that obtained by measuring the area under the concentration curve constructed by plotting the level in multiple blood samples taken at short intervals. The method for the constant blood withdrawal, permitting the determination of integrated plasma concentration, has been described previously.[30] A small portable pump, which draws blood at a constant rate through a nonthrombogenic i.v. catheter, is strapped to the chest of the subject. The IC is obtained in subjects as they pursue normal activity, encompassing periods of lying, standing, and walking.

MATERIALS AND METHODS

Experimental subjects. Forty-five experimental subjects participated in the study: 34 of them were normal control subjects who had no detectable endocrine abnormalities and who ate freely. Eleven subjects had insulin-dependent diabetes. The patients were within 15% of ideal body weight for their height[31] and were studied after an overnight fast. All

received their daily insulin dose at between 0800 h and 0900 h. No patient had ketonuria at the time of the study. The day's total urinary glucose excretion of the patients was determined when they were eating a standard ADA diet for their height, weight, and age. All subjects were encouraged to pursue a normal level of activity. All the diabetic patients and normal control subjects were studied under similar conditions of activity in the same clinical research unit. Meal patterns were similar. Carbohydrate intake of the normal subjects was 45% and of the diabetic patients, 45%.

Laboratory methods. PRA, plasma ALDO, F, and growth hormone (GH) were measured by previously described methods.[32–35] E and NE concentrations in the plasma were measured using a radioenzymatic assay.[36,37] Blood samples were collected continuously using the previously described nonthrombogenic, constant, blood withdrawal system.[30] This method has been used in the past for measuring the IC of GH,[30] PRA and ALDO,[38] F,[29] and other hormones whose concentration fluctuates rapidly during the day. The test tubes in which the blood was being collected were replaced at half-hour intervals. The plasma was separated and frozen at the end of each half-hour collection. After plasma separation, aliquots were taken from each 30 min collection and combined, producing a 24 h plasma pool in which the IC-ALDO, IC-PRA, IC-NE, IC-E, IC-F, and IC-GH were measured. The stability of F, ALDO, and PRA during blood withdrawal has been studied and reported previously.[24,29,39] The stability of NE and E at room temperature, determined by use of a previously described[40] stabilizing solution, was measured by assaying the level of NE and E in 16 blood samples immediately after withdrawal and again at the end of 3 h at room temperature. The effect of blood transit through the catheter before mixing with the stabilizing solution was tested as described below.

The day-to-day variability of the tests was evaluated in 20 normal subjects by repeating the tests 2 wk after the initial procedure. Correlation between the results measured at 2 wk intervals was analyzed by linear regression analysis. The variability of each laboratory test was evaluated by the variance ratio method.

RESULTS

Stability of norepinephrine and epinephrine. The level of NE was 360.4 ± 224.9 pg/ml immediately after withdrawal and 352.6 ± 169.3 at the end of 3 h at room temperature. The $\bar{x}$ ± 1 SD level of E was 73.5 ± 75.1 pg/ml before and 64.2 ± 75.1 at the end of 3 h at room temperature. The difference between the two measurements was not significant. The effect of heparin on the stability of NE and E was studied by adding heparin to the freshly withdrawn blood and measuring the level of NE and E upon withdrawal after 15 min at room temperature. The concentration of NE was 210 ± 61 pg/ml at the time it was withdrawn and 187.5 ± 74 pg/ml after 15 min at room temperature. The level of E was 60.6 ± 11 pg/ml upon withdrawal and 57.4 ± 8 at the end of 15 min. The effect of heparin on the stability of NE and E was not significant. The transit time of blood in the catheter never exceeded 10 min. It is thus apparent that no significant lowering of the concentration of NE and E occurred during that period of time.

Table 2 depicts the correlation between results repeated at 2 wk intervals in the same subject. The correlation was significant ($P < 0.02$) for each test. The $\bar{x}$ ± 1 SD of age, weight, blood pressure, urine volume, 24 h glucose excretion, and the IC of NE, E, PRA, ALDO, F, and GH, are given in Table 1. IC-NE and IC-E are shown in Figure 1. There was no significant difference in the ages, weights, blood pressure, IC-PRA, and IC-F between the two groups studied, while the mean of IC-ALDO, IC-NE, IC-E, and IC-GH was significantly higher in the diabetics than in the control subjects. There was no significant correlation between IC-glucose and the IC of NE, E, PRA, ALDO, F, and GH.

TABLE 1
Pertinent clinical and laboratory data

	Subjects		Significance of the difference
	Controls	Diabetics	
Number	34	11	
Age (yr)	15.4 ± 3.6	16.0 ± 3.2	NS
Wt (kg)	57.1 ± 15	61.3 ± 19	NS
Blood pressure (mm Hg)			
Systolic	115 ± 17	116 ± 16	NS
Diastolic	71 ± 8	78 ± 7	NS
IC-glucose (mg/dl)	—	299 ± 81	—
Urine glucose (g/24 h)	0	96 ± 79	—
Urine volume (L/24 h)	1.1 ± 0.3	2.8 ± 1.5	$P < 0.001$
Urine potassium (mEq/24 h)	49 ± 22	81.2 ± 169	NS
Urine sodium (mEq/24 h)	137 ± 64	168 ± 40	NS
Plasma potassium (mEq/24 h)	3.9 ± 0.4	4.3 ± 0.4	NS
Plasma sodium (mEq/24 h)	142 ± 3	139 ± 4	NS
IC-norepinephrine (NE) (pg/ml)	148 ± 74 (60–302)	240 ± 81 (161–413)	$P < 0.01$
IC-epinephrine (E) (pg/ml)	26 ± 13 (12–62)	37.6 ± 7 (30–50)	$P < 0.02$
IC-aldosterone (ALDO) (ng/dl)	12.5 ± 4.7 (4.4–22.3)	20.4 ± 6.8 (7–32)	$P < 0.0001$
IC-plasma renin activity (PRA) (ng/ml/h)	0.8 ± 0.4 (0.3–1.9)	0.9 ± 0.6 (0.1–1.9)	NS
IC-cortisol (F) (mcg/dl)	5.5 ± 1.9 (2.2–11.9)	6.6 ± 1.8 (4.9–11.4)	NS
IC-growth hormone (GH) (ng/ml)	4.3 ± 2.3 (1.3–8.9)	11.3 ± 6.4 (6.4–22.5)	$P < 0.0001$

DISCUSSION

Previous studies on the plasma levels of NE, E, and ALDO in diabetic patients were based on measuring the concentration in discrete plasma samples. The discrepant results may be attributed to the wide and rapid fluctuation of the plasma levels of these hormones.[23–26] Thus, a single discrete sample of blood may have been taken during a peak or a nadir of fluctuating plasma level, greatly increasing the variability and obscuring the difference between normal subjects and the diabetic patients.

Significant changes in the average 24 h concentration could, therefore, go undetected if only a single or a small

TABLE 2
Correlation of the IC studies in 20 normal individuals repeated at 2 wk intervals in the same subject

	IC-PRA	IC-ALDO	IC-F	IC-NE	IC-E	IC-GH
R	0.447	0.856	0.751	0.697	0.800	0.784
P <	0.02	0.001	0.001	0.01	0.001	0.001

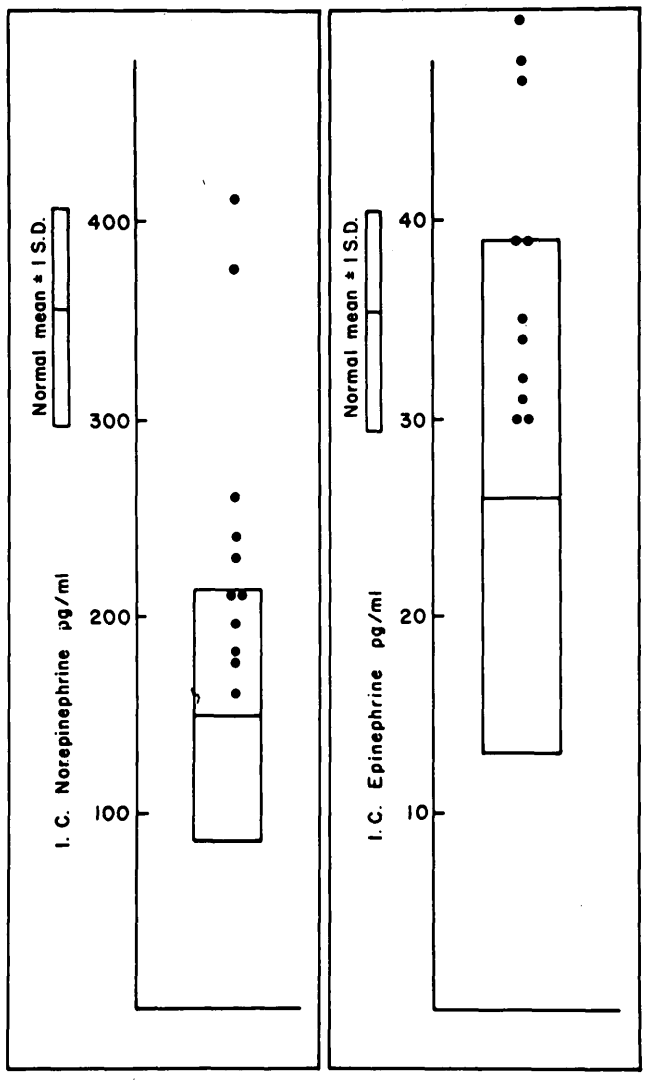

FIGURE 1. The IC-norepinephrine and IC-epinephrine of the patients in relation to the $\bar{x} \pm 1$ SD of these values in normal subjects.

number of samples is obtained. IC is defined as the concentration of a substance in a pool of blood collected by withdrawal at a constant rate from a peripheral vessel. The result of such a determination is somewhat similar, although it is more accurate than that obtained by measuring the area under the concentration curve constructed by plotting the level in multiple blood samples taken at short intervals. The method for the constant blood withdrawal, permitting the determination of integrated plasma concentration, has been previously described.[30] In our study, we measured the integrated 24 h blood concentration of patients and compared the results with normal age-matched control subjects. By integrating the plasma level, we were able to cancel the effect of fluctuation, thereby lowering the variability and the range of the results.[40]

Our observation of an increased integrated plasma level of NE and E in hyperglycemic juvenile diabetic patients who had no ketosis agrees with previous studies by Halter et al.[4] and Robertson et al.[5]

Our observation of a significant elevation of the IC of ALDO, without a concomitant increase in the IC-PRA, is also of interest. While some previous observations suggested an abnormality of the renin-angiotensin-aldosterone system in patients with long-standing complicated diabetes,[8–17] other investigators reported normal levels of PRA and ALDO.[18–22] Moss et al.[22] felt that hyporeninemic hypoaldosteronism is a consequence of complications associated with long-standing disease. It should be noted that we studied young patients whose diabetes was of relatively recent onset.

Since the increased plasma level of ALDO was not associated with an increase in PRA, its major trophic hormone, the increased ALDO level should be attributed to another stimulus. It is difficult to attribute the elevation of plasma ALDO to an increased level of ACTH, since the IC-F in the diabetic patients was normal. Likewise, the elevation of IC-ALDO could not be accounted for by a hyperglycemia-induced rise in plasma potassium, although plasma aldosterone concentration does increase with potassium administration sufficient to cause only a trivial increase in the concentration of potassium in plasma.[41] We found no significant difference between the plasma potassium concentration or the urinary excretion of potassium of our diabetic patients and the control subjects.

The IC of a hormone does not correspond directly with its secretion rate. For example, because of individual variations and frequent changes in the metabolic clearance of ALDO, the maintenance of the same IC-ALDO may require different secretion rates in different individuals.[42–44] A decrease in the metabolic clearance rate of ALDO is, therefore, a possible mechanism for our observations. Previous studies have reported increased plasma concentration of ALDO associated with increased PRA in diabetic patients with ketoacidosis.[45] In nonketotic patients, it was postulated that hyperglycemia would cause hypervolemia and decrease the PRA and the level of PRA and ALDO.[17] Our results disagree with this postulate. Hyperaldosteronism could be secondary to sodium depletion owing to the diuretic effect of the glycosuria. However, since our patients did not exhibit a concomitant elevation of PRA, secondary hyperaldosteronism is unlikely.

Our observation of an increased IC-GH in the insulin-dependent juvenile-onset diabetic patients agrees with a previous report from our group.[45] The reason for the association is not clear. Since GH and the catecholamines are part of the counterregulatory hormones' response to hypoglycemia, their levels in hyperglycemic patients should have been low rather than high.

The mechanism underlying our observation of an increased level of NE, E, and GH in our patients cannot be determined from our studies. We can only speculate that the increase is a response to postulated rapid changes in the plasma level of glucose which occurred in these patients. It should be pointed out that, even though all our patients were treated with insulin, the dose was insufficient; thus, the hyperglycemia and glycosuria. We can assume that each administration of insulin must have been followed by a period of rapid lowering of the plasma level of glucose, and a subsequent rise, back to hyperglycemic levels. The increased concentration of three of the counterregulatory hormones may have occurred during periods of insulin-induced rapid lowering of the glucose level. It is of interest that we have not observed a concomitant elevation in the IC-F, even though F levels do increase during insulin-induced hypoglycemia.[46] We can speculate that the hypoglycemic

stimulus required by F is greater than the stimulus for GH, NE, and E. It should be noted that Clarke et al.[47] reported an increase in plasma NE, E, and GH, but no increase in plasma F, during a lowering of plasma glucose from 95 to 60 mg/dl in diabetic patients and in normal subjects.

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