C-Peptide Secretion During the Remission Phase of Juvenile Diabetes

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SUMMARY

C-peptide secretion was studied in eight juvenile diabetics during the remission phase of the disease. The release of C-peptide was measured after a (1) normal intravenous glucose tolerance test, (2) a double glucose tolerance test, (3) an arginine infusion, and (4) after an intravenous glucose tolerance test followed by an arginine infusion. Under all conditions the intravenous glucose load had only a minimal effect on the secretion of C-peptide, while arginine alone or after the intravenous glucose tolerance test stimulated the release of the peptide in all patients. Pretreatment with glucose did not augment the effect of arginine on C-peptide release.

The results indicate that during the remission phase of juvenile-onset diabetes the endocrine pancreas does not recognize glucose as an appropriate signal for C-peptide release and cannot transform the amplifying effect of glucose into a higher hormonal secretion rate. DIABETES *27*:670-76, June, 1978.

Juvenile-onset diabetes is characterized by a complete insulin deficiency.[1] Shortly after onset of the disease and after an appropriate treatment, a substantial number of patients recover from the absolute exogenous insulin dependence and a partial or complete remission of the disease can occur.[2-6] The duration of this "honeymoon" period of juvenile diabetes may last from a few weeks to several months or even years during which time the patients can be effectively treated with diet alone or with diet and small amounts of insulin.[7,8] Recently Pirart and Lauvaux[9] reviewed the current knowledge about the remission phase of diabetes mellitus. The dynamics of insulin release during the remission phase of diabetes are still poorly understood, though the effect of different stimuli such as glucose, tolbutamide, glucagon, and a mixed meal has been tested with somewhat conflicting results.[10-12] The studies are partly hampered by the fact that there is no method available to distinguish between the endogenously secreted and the exogenously administered insulin. The development of C-peptide radioimmunoassays overcomes this difficulty when changes in the secretion rate are measured.[13-15] C-peptide is released in equimolar amounts with insulin from the B-cells and may be taken as an indicator for the capacity of the pancreas to liberate insulin.[16]

The aim of the present study was to further characterize the B-cell function during the remission phase of juvenile diabetes by measuring the serum C-peptide concentration after various stimuli.

MATERIAL AND METHODS

Patients. The pertinent clinical data of the eight patients are presented in table 1. Three male and five female juvenile diabetics between seven and 21 years of age were studied. They all had a normal or slightly decreased body weight, and their heights were within the normal range. The date of diagnosis, the blood sugar values when the patients arrived at the clinic, and the initial insulin treatment as well as the current therapy (1977) are presented. The dates when the different tests were performed are also shown. Seven healthy medical students with no family history of diabetes mellitus served as controls.

The patients and the parents of the children were fully informed about the purpose of the tests. The character of the merely investigative study was emphasized.

Tests. All tests were performed between 0800 and

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TABLE 1

Clinical data of the eight juvenile diabetics

Patient	Sex	Age at onset (years)	Date of diagnosis	Date of study	Test	K-values	Insulin therapy at test (U./day)	Initial clinical data	Current therapy: morning, evening
1	F	13	6-74	11-74	GA	1.14	—	BS: 1,100 mg./dl.	8† + 18‡
				12-74	GA	1.46	—	Insulin:* 100 U. s.c. + 100 U. I.V.	6 + 12
				2-75	A				
2	M	11	1-75	2-75	GA	0.61	—	BS: 722 mg./dl.	10 + 22
				5-75	A		—	Insulin: 100 U. s.c. + 100 U. I.V.	6 + 16
3	F	7	10-75	1-76	GA	0.97		BS: 340 mg./dl.	4 + 8
				1-76	A		4+2 NPH	Insulin per day: 3 × 8 U. s.c.	2 + 4
4	F	14	2-76	2-76	GG	0.62; 0.61	6+2 NPH	BS: 440 mg./dl. Insulin per day: 3 × 14 U. s.c.	6 + 16 2 + 4
5	F	8	2-76	3-76	A		—	BS: 220 mg./dl.	2 + 4
				4-76	GG	0.68; 0.61	—	Insulin per day: 3 × 8 U. s.c.	—
6	M	10	12-74	1-75	A		6 NPH	BS: 1,158 mg./dl.	12 + 20
				1-75	GG	0.71; 0.69	—	Insulin: 50 U. s.c. + 50 U. I.V.	4 + 10
7	F	21	6-74	8-74	GA	0.79	—	BS: 280 mg./dl.; diet + 5 mg.	16 + 26
				8-74	GG	0.63; 0.58	—	sulfonylurea per day	8 + 14
8	M	9	5-75	5-75	GG	0.98; 1.20	—	BS: 89 mg./dl.; pathologic OGTT; diet	10 + 22 6 + 16

GA, intravenous glucose tolerance test followed by an arginine infusion; A, arginine infusion; GG, double intravenous glucose tolerance test. At the beginning of therapy the patients received *regular insulin, while the current therapy is achieved with a combination of †regular insulin plus ‡NPH.

0900 hours after an overnight fast of 12 to 14 hours. Patients 3, 4, and 6 (table 1) had received their last insulin injections 24 hours before the corresponding test, while the remaining five patients were without any insulin treatment for at least one week.

During the arginine test (A) 0.5 gm. per kilogram body weight of arginine hydrochloride as a 10 per cent solution was infused over 30 minutes. At 0, 15, 30, 45, and 60 minutes after the beginning of the infusion, blood was withdrawn for the determination of the blood glucose and the serum immunoreactive C-peptide concentrations (see below). The test was performed in five patients and the seven controls.

The glucose-arginine test (GA) was done with 0.33 gm. per kilogram of glucose as a 40 per cent solution, which was injected within two minutes. Sixty minutes after the administration of the glucose load an arginine test was immediately started under the same conditions as outlined above. At the indicated time intervals, blood was collected (see figures) for the measurement of glucose and the immunoreactive C-peptide. Both parameters were again determined in four patients during a double intravenous glucose tolerance test (GG). During the first as well as during the second test the patients received 0.33 gm. per kilogram of glucose as a 40 per cent solution I.V. The time interval between the two glucose injections was 60 minutes.

Determination of Glucose and C-Peptide

Blood glucose was determined in duplicate with an AutoAnalyzer (Technikon, Bad Vilbel, West Germany) according to the GOD-Period method.[17,18] The K-values were calculated according to Conard.[19] The C-peptide (IMCP) was measured by radioimmunoassay as described previously. The C-peptide antibody cross-reacts with human proinsulin but not with insulin.[15] The results are expressed in molarity which is based on the calculation that 1 ng. IMCP per milliliter serum is equal to 33.06 moles × 10^{-11} IMCP per liter serum.

Calculations. Comparisons were made between the IMCP peaks which were reached during the different tests. The results are presented as Δ peak and denote the means ± S. E. of the means. Student's paired *t*-test was used when comparisons were made within the same test while the degrees of significance between the different tests were calculated with the unpaired student's *t*-test.

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RESULTS

Patients. The histories of the eight patients, except that of child 8, were all suggestive for diabetes mellitus with sudden onset of clinical symptoms three to six weeks before the diagnosis was finally established. In patient 8 (table 1) an appendectomy was performed. During the routine urine examination glucosuria was detected. At that time the fasting blood sugar concentrations ranged between 160 and 180 mg. per deciliter. Three weeks after the operation the oral glucose tolerance test was grossly abnormal, and he was transferred to our clinic for further treatment.

The initial clinical data of the eight patients are summarized in table 1. Treatment was immediately initiated as soon as the diagnosis of diabetes mellitus was confirmed. The therapeutic aim was to keep the patients' urine free of glucose and at the same time avoid hypoglycemic attacks. This could be achieved during the remission phase in three patients with small amounts of an intermediate insulin preparation. The remaining five patients were treated with diet alone. At present all patients receive a mixture of a regular and an intermediate insulin preparation twice a day (table 1).

Tests. The conventional arginine tolerance test was performed in five patients. Figure 1 shows the results. Patient 6 had a fasting blood sugar of 131 mg. per deciliter, while the glucose concentrations of the four remaining patients were within the normal range. At 30 to 45 minutes after the beginning of the arginine infusion, the peak glucose level was reached, except in patient 6, in whom a constant increase of the blood sugar was observed during the whole test period. The fasting serum of the five patients contained immunoreactive C-peptide. In all patients arginine stimulated the secretion of the C-peptide from the B-cells. The increase in immunoreactive C-peptide was 34.6 ± 8 moles × 10^{-11} IMCP per liter or 52.6 ± 9.9 per cent compared with zero minutes, $p < 0.001$. In the seven normal controls a Δ peak of 84.5 ± 8.8 moles × 10^{-11} IMCP per liter or an increase of 199.5 ± 31.3 per cent was reached by the infusion of arginine.

In five patients, five combined glucose-arginine tests were done. The K-values (table 1) were abnormal during four intravenous glucose tolerance tests, while patient 1 had a normal K-value of 1.46 at the second examination. During the arginine tests, which were started 60 minutes after the glucose injection, the blood sugar concentrations remained unchanged or

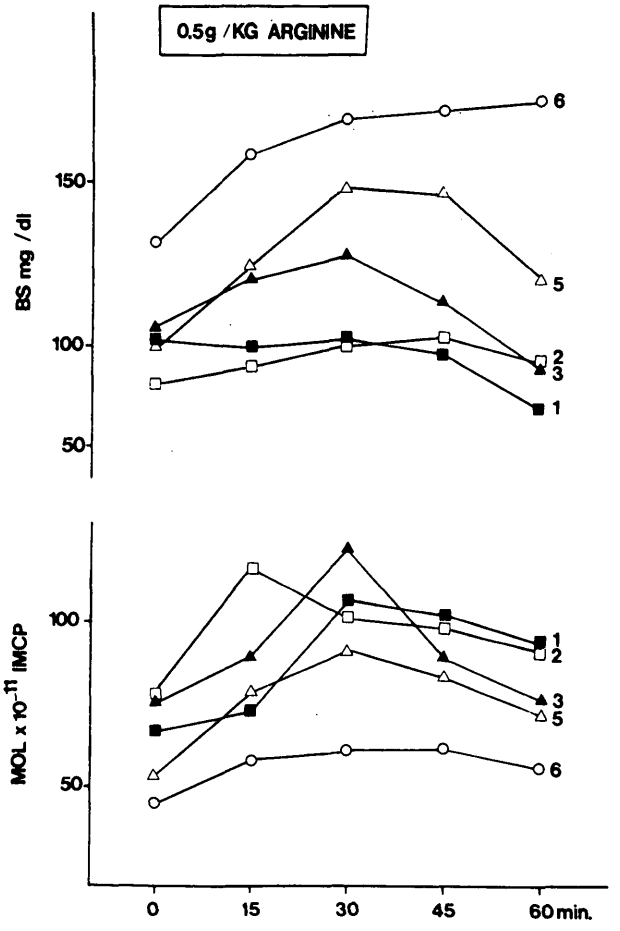

FIG. 1. Effect of an arginine infusion on the blood sugar (BS) and the immunomeasurable C-peptide (IMCP) concentrations in five juvenile diabetics during the remission phase. The increase of the C-peptide concentration calculated as Δ peak vs. zero minutes was 34.8 ± 8 moles × 10^{-11} IMCP per liter.

decreased in all patients. Figure 2 shows the pattern of the blood glucose and the C-peptide secretion during the intravenous glucose tolerance tests and the following arginine infusions. Glucose had only a minimal effect on the release of the C-peptide, with an increase of 15.7 ± 3.6 moles × 10^{-11} IMCP per liter or 16 ± 4 per cent compared with zero minutes, $p < 0.02$. Arginine augmented the secretion in all patients. The peak amounted to 50.4 ± 11.9 moles × 10^{-11} IMCP per liter or 52 ± 13.6 per cent, $p < 0.02$, as against the start of the amino acid infusion. In comparison with the results of the conventional arginine tests the preceding glucose injections did not modify the release of the C-peptide during the subsequent arginine infusions: Δ peak of the arginine test was 34.6 ± 8 vs. 50.4 ± 11.9 moles × 10^{-11} IMCP per liter as the increment during the glucose-arginine test.

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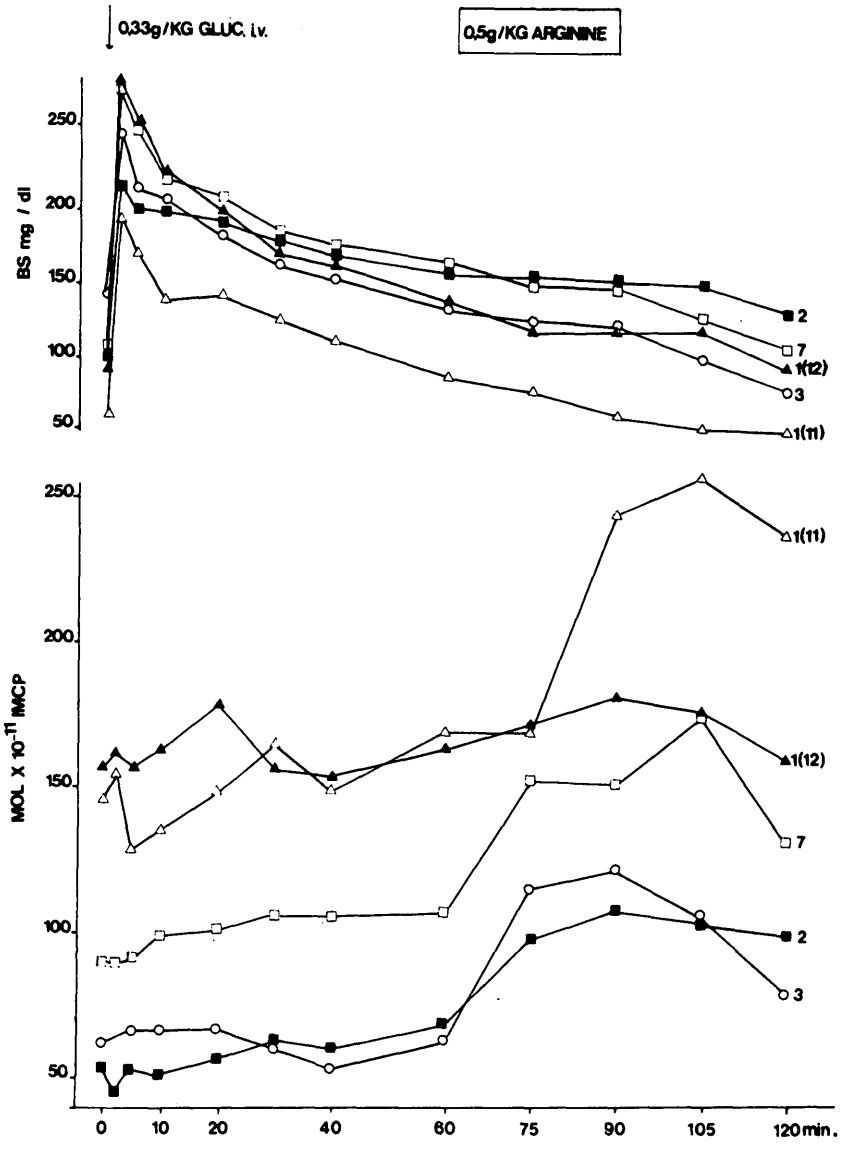

FIGURE 2

Blood sugar (BS) and immunomeasurable C-peptide (IMCP) concentrations during the intravenous glucose tolerance test and the subsequent arginine infusions in five juvenile diabetics during the remission phase. Glucose tolerance test: zero minutes vs. Δ peak, 15.7 ± 3.6 moles × 10^{-11} IMCP per liter; $p < 0.02$. Arginine infusion: 60 minutes vs. Δ peak, 50.4 ± 11.9 moles × 10^{-11} IMCP per liter; $p < 0.02$.

The double intravenous glucose tolerance test was performed in four patients. The K-values are presented in table 1. The K-values during both tests were abnormal in all patients. They did not differ during the first or the second test, except in patient 8. The K-value of the first glucose tolerance test was 0.98 and was almost normal during the second examination at 1.20. This boy was treated with diet alone. The blood glucose and the C-peptide concentrations of the double intravenous glucose tolerance tests are shown in figure 3. During the first and second glucose load equal small amounts of C-peptide were liberated from the B-cells. The Δ peak reached, in the first test, 16.5 ± 5 and, in the second test, 13.5 ± 4 moles × 10^{-11} IMCP per liter.

DISCUSSION

Insulin and C-peptide are secreted from the B-cell in equimolar amounts.[14,16,20] By measuring C-peptide instead of insulin it became possible to determine the secretory capacity of the endocrine pancreas in insulin-treated diabetic patients, since exogenously administered insulin does not disturb the C-peptide radioimmunoassay. Human proinsulin, which is released by the islets of Langerhans, is recognized by antibodies raised against human C-peptide and is determined to be immunomeasurable C-peptide.[13-15,21,22] In normals as well as in diabetics, however, the serum contains less than 5 per cent of proinsulin,[13,16,23,24-26] except in a few insulin-

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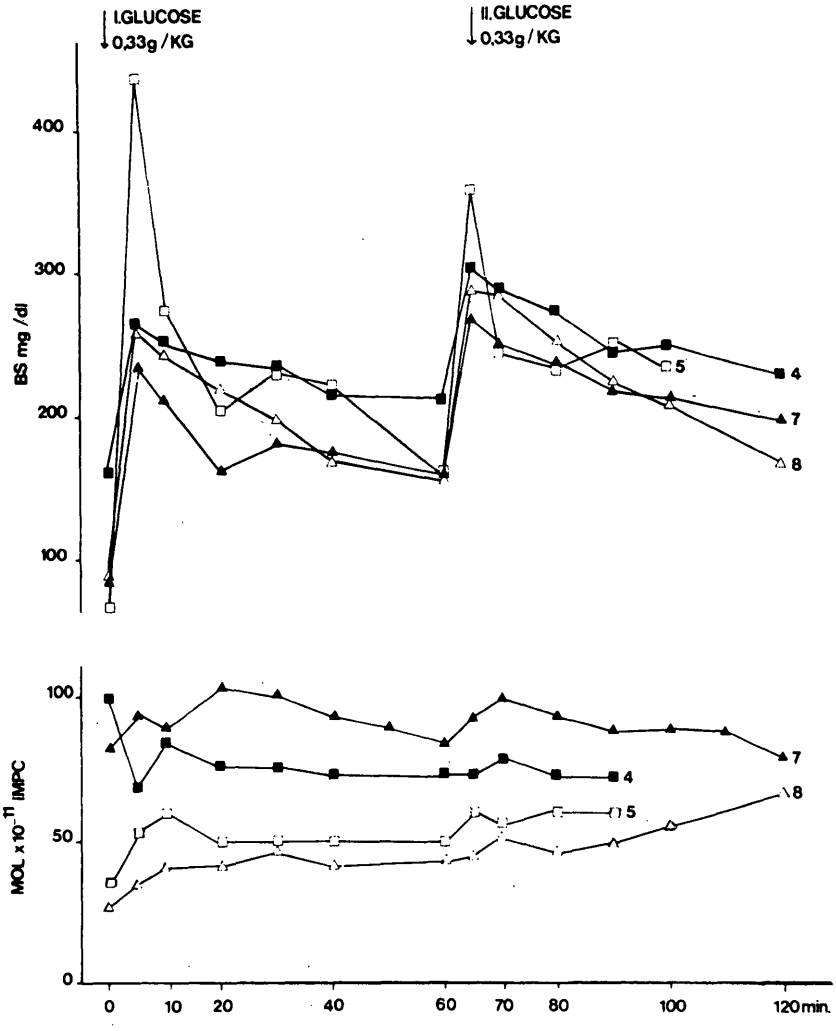

FIGURE 3

Blood sugar (BS) and immunomeasurable C-peptide (IMCP) concentrations during the double intravenous glucose tolerance test in four juvenile diabetics during the remission phase. (1) Test: Δ peak 16.5 ± 5 moles × 10^{-11} IMCP per liter. (2) Test: Δ peak 13.5 ± 4 moles × 10^{-11} IMCP per liter.

treated diabetic patients in whom extremely high serum IMCP and proinsulin concentrations were found.[27,28]

Several reports in the literature deal with basal and stimulated insulin concentrations in juvenile diabetics either before any treatment had been initiated or during the remission phase of the disease. In general, at the beginning of overt juvenile diabetes the serum contained no or low insulin or C-peptide concentrations, and various stimuli such as glucose, glucagon, tolbutamide, and arginine had no effect on the secretion of the hormone from the B-cells.[1,10-12,29] During the remission phase of the disease, however, the basal and, in some patients, the stimulated insulin release had greatly improved.[2-6] More recently the effect of an oral glucose tolerance test on the release of C-peptide has been reported. During severe ketoacidosis no liberation could be obtained, while after strict treatment and a remarkable improvement of the diabetic state, glucose greatly stimulated secretion of the C-peptide from the islets of Langerhans in those patients who had a normal fasting blood sugar and a normal or almost normal glucose tolerance test.[26] In our patients the intravenous glucose load had only a minimal effect on C-peptide release despite a normal fasting blood glucose concentration during the majority of the tests. Therefore a comparison with the above cited patients of Block and co-workers appears to be justified.[26] The results these authors obtained strongly suggest that during the remission phase of juvenile diabetes mellitus the B-cells recognize gut factors as appropriate signals for C-peptide release, while the glucose-dependent mechanisms for C-peptide secretion are impaired as shown by the almost complete failure of the venous glucose load to stimulate the liberation of C-peptide. Furthermore, in

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the present study it has been shown that there exists a clearcut difference between the effect of glucose and arginine on the secretion of C-peptide during the remission phase in juvenile diabetics.

The serum C-peptide secretion was only slightly stimulated by a single or a double intravenous glucose load. In contrast, arginine alone, and after a preceding intravenous glucose tolerance test, enhanced the secretion of C-peptide from the islets of Langerhans. However, the response of C-peptide to amino acid was significantly lower than in the control subjects.

The results differ from those that were obtained in normal,[30] prediabetic, and mildly diabetic subjects.[31] In all three groups two consecutive glucose infusions, administered at a comparable time interval with the present study, resulted in a higher insulin secretion during the second test. The low insulin release in the prediabetics was normalized by their pretreatment with glucose, and in the mild diabetics hormonal secretion was greatly improved. It was concluded that the sensitivity of the B-cells for the potentiation of glucose on insulin secretion was not different from that of the controls.[31]

Our results indicate that in the remission phase of juvenile-onset diabetes the B-cells neither recognize glucose as an appropriate signal for C-peptide release nor can they transform the amplifying effect of a preceding glucose load into a higher hormonal secretion rate. The conclusions are further supported by the observation that, in our patients, arginine stimulated the secretion of C-peptide from the endocrine pancreas, and pretreatment with glucose did not modify this response. In normals, however, a glucose load augmented the insulin release during a subsequent arginine tolerance test.[32] Furthermore, ten noninsulin-requiring diabetics (with fasting blood sugars above 125 mg. per deciliter) showed unimpaired insulin secretion after an arginine infusion. The patients were treated with diet alone.[33]

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