Diabetologia 11, 125–128 (1975)

The Effect of Phenformin on Amino Acid-Induced Insulin Secretion in Diabetics*

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Received: August 15, 1974, and in revised form: December 12, 1974

Summary. Twenty-one patients with mild maturity-onset diabetes were given introduodenal infusions of an amino acid mixture (0.5 g amino acids per kg body weight). In 9 other patients L-arginine was infused intravenously in a constant dose of 25 g. Alpha-amino nitrogen, blood glucose and plasma insulin levels were assayed under control conditions and after three days of treatment with phenformin, 150 mg daily, plus the same 150 mg dose 60 min before the second loading. Intraduodenal infusion of the amino acid mixture provoked a greater increase in plasma insulin than intravenous infusion of L-arginine, this increase being significantly inhibited by phenformin only in the first case. Since no evident influence of phenformin on the intestinal absorption of amino acids could be demonstrated, this effect may be explained by a local action on the intestinal wall exposed to high concentrations of the drug, resulting in the inhibition of the insulin secretion stimulating activity of the gut.

Key words: Diabetes, antidiabetic biguanides, amino acids, L-arginine, blood glucose, plasma insulin, intraduodenal amino acid administration.

Phenformin is known to reduce excessive levels of serum insulin after glucose loading in obese nondiabetic subjects, as well as in patients with maturity-onset diabetes [2]. However, this suppression by phenformin, which is distinct when glucose is administered orally, does not occur after intravenous infusion [3]. Since the secretion of insulin is also stimulated by amino acids [4], in this study we have compared the effect of phenformin on the secretion of insulin in patients with mild diabetes, to whom amino acids were administered intraduodenally as well as intravenously.

Material and Methods

30 patients were studied, having maturity-onset diabetes of 1–6 years duration, satisfactorily treated with diet only. The group included 18 men and 12 women aged 42–68 years. Fourteen patients were 14–28% and one 40 per cent above ideal body weight. In 21 patients, a solution of an amino acid mixture (Polfa, Poland[1]) was infused intraduodenally in doses of 0.5 g of amino acids per kg body weight. This dose of amino acids has been shown to cause an elevation in plasma alpha-amino nitrogen, which returned to the starting values after 3 hrs. The amino acid solution was infused over 15 minutes without causing any signs of intolerance. In 9 other subjects an aqueous solution of 25 g L-arginine (Laboratoire Fermé, France) was infused intravenously over 30 min. A control test was always followed by a second test performed after 3 days of administration of phenformin, 150 mg daily; the same dose was given on the day of the second test one hour before administering amino acids or L-arginine. Alpha-amino nitrogen was assayed in plasma by the method described by Frame, Russell and Wilhelmi and modified by Russell [5–6], and blood glucose was assayed by the method of King. Insulin was measured in heparinized plasma by radio-immunoassay [7]. Since each subject served as his own control, the effect of phenformin on the various blood levels was assessed by the paired 't' test, for significance level $p < 0.05$.

Results

Intraduodenal Infusion of Amino Acid Solution

Intraduodenal infusion of the mixture of amino acids caused a significant increase in plasma levels of alpha-amino nitrogen, which were highest 30–60 min after beginning the infusion and returned to the starting level towards the end of the test (see Table 1). In 15 of 21 patients, administration of phenformin was followed by a rise in fasting plasma values of alpha-amino nitrogen, and this exerted an influence on the mean value for the whole group. After intraduodenal infusion of the amino acid mixture, elevation of plasma

* Part of this work was presented at the 7th Congress of IDF in Buenos Aires, Argentina, 1970 /1/.

[1] Composition of the amino acid mixture in g per litre: L-arginine 2.90; L-aspartic acid 3.00; L-cystine 0.88; L-phenylalanine 3.30; DL-glutamic acid 3.20; L-histidine 3.00; L-isoleucine 1.80; L-leucine 7.49; L-lysine 7.22; L-methionine 1.15; L-proline 2.26; L-serine 3.39; L-threonine 1.82; L-tryptophan 0.56; L-tyrosine 0.83; L-valine 4.40.

Table 1. *The effect of phenformin on the plasma amino nitrogen, blood glucose and plasma insulin levels after intraduodenal administration of amino acid solution in diabetic patients*

Time (min)	Plasma Amino-N (mg/100ml) n=21				Blood Glucose (mg/100 ml) n=21				Plasma Insulin (IRI) (μU per ml) n=15			
	Before	After	d (After-Before)	p	Before	After	d (After-Before)	p	Before	After	d (After-Before)	p
0	5.0±0.16[a]	5.4±0.13[a]	+0.44±0.15[a]	<0.01	139.8±8.3[a]	127.6±8.4[a]	−12.19±4.63[a]	<0.01	16.9± 2.8[a]	17.7±2.4[a]	+ 0.13± 2.31[a]	NS
15	6.1±0.18	6.3±0.13	+0.15±0.18	NS	144.8±8.2	128.0±8.5	−16.76±3.84	0.0005	60.9±14.2	26.5±4.4	−33.43±12.19	<0.01
30	6.8±0.25	7.0±0.17	+0.20±0.27	NS	141.1±8.2	124.6±5.8	−16.57±4.45	0.0025	52.6±12.8	22.5±4.3	−28.64±11.13	<0.025
45	6.8±0.22	7.0±0.18	+0.35±0.27	NS	142.6±8.4	120.6±8.0	−21.40±4.29	0.0005	43.6±10.8	16.0±2.6	−26.00±11.40	<0.025
60	6.6±0.24	6.7±0.17	+0.09±0.26	NS	140.0±7.9	122.0±8.4	−18.38±4.80	0.0025	23.6± 4.8	19.4±4.1	− 5.08± 2.84	NS
90	6.1±0.25	6.2±0.15	−0.02±0.29	NS	138.6±8.4	117.6±8.3	−23.58±3.46	0.0005	15.6± 2.8	15.2±2.4	− 0.46± 2.09	NS
120	5.7±0.20	5.8±0.17	+0.22±0.25	NS	134.2±8.3	115.6±8.7	−18.60±4.50	0.0005	18.9± 3.1	17.0±4.2	− 2.45± 4.06	NS
150	5.4±0.08	5.5±0.17	+0.07±0.28	NS	132.5±7.4	109.6±8.5	−22.80±5.42	0.0005	17.6± 5.7	10.7±3.8	− 4.75± 7.82	NS
180	4.9±0.16	5.4±0.16	+0.44±0.15	<0.01	124.6±6.3	105.6±6.7	−21.89±4.34	0.0005	10.2± 3.3	12.0±7.0	+ 3.33± 6.26	NS

[a] SEM

alpha-amino nitrogen in 11 of 21 patients was smaller in patients taking phenformin than in the control tests. However, this difference was not significant in the entire group. Intraduodenal infusion of amino acids caused a small increase in the blood sugar level, lasting up to 60 min, followed by a slight fall. After phenformin treatment, the levels of blood glucose were significantly lower both on fasting and during the whole test (see Table 1). In 14 of 21 patients the decrease in glycemia induced by the amino acid load was greater, compared with the control test. The mean decrease in glycemia at the end of the test, calculated as a percentage of the starting value, was significantly greater after phenformin than in the control tests (at 180 min. $p<0.0125$).

The behaviour of plasma insulin after intraduodenal infusion of the amino acid solution was examined in 15 patients. In all, the control tests showed a distinct increase in plasma insulin levels, which in some cases was considerable, exceeding 100 μU per ml. The highest plasma levels of insulin were noted at 15 and 30 min from the start of the intraduodenal infusion, returning to the starting values after 60 and 90 min. After prior phenformin treatment, the increase in insulin levels following the intraduodenal infusion of amino acids was slight, and the differences between the mean values of plasma insulin at 15, 30 and 45 min of this test, and analogous values of the control test, were statistically significant (see Table 1).

Intravenous Infusion of L-Arginine

Intravenous infusion of 25 g of L-arginine caused a small increase in glycemia at the beginning followed by a slight decrease at a later stage of the test (see Table 2). In one patient, however, after infusion of L-arginine the blood glucose dropped to 66 mg% in the course of one hour. Treatment with phenformin resulted in somewhat lower values of fasting glycemia, and the increase in blood glucose levels after infusion of L-arginine was somewhat greater. In these subjects, intravenous infusion of L-arginine proved to be a weak stimulus for insulin secretion. The increase in insulinemia was small, but occurred in all cases except one. Prior treatment with phenformin did not alter the course of insulinemia after intravenous infusion of L-arginine.

Discussion

Convincing evidence from *in vitro* and *in vivo* studies with animals and in man has been presented showing that antidiabetic biguanide derivatives inhibit intestinal absorption of glucose [8—9], galactose [10],

amino acids [11], vitamin B_{12} [12], and sodium and water [13–14]. Our data indicate that phenformin does not exert any significant effect on the course of the plasma amino nitrogen curve following intraduodenal administration of an amino acid mixture in man. Since Caspary and Creutzfeldt have shown that the blood sugar lowering biguanides distinctly inhibit sulinemia after intravenous infusion of L-arginine in obese diabetics. There is an obvious analogy with the influence of phenformin on blood levels of insulin after oral and intravenous administration of glucose [2]. The fact that phenformin had only a very small influence on plasma alpha-amino nitrogen levels indicates that the inhibition of the increase in insulinemia is not

Table 2. *The effect of phenformin on the blood glucose and plasma insulin levels after intravenous infusion of L-arginine solution in diabetic patients*

Time (min)	Blood Glucose (mg/100 ml) n=8				Plasma Insulin (IRI) (μU per ml) n=9			
	Before	After	d (After-Before)	p	Before	After	d (After-Before)	p
0	128.5±4.8[a]	116.7±5.9[a]	−11.75± 4.20[a]	<0.025	18.8± 4.8[a]	19.3±4.9[a]	+ 0.56±5.69[a]	NS
10	133.3±8.1	127.3±5.9	− 6.00± 7.24	NS	19.0± 2.5	18.7±3.9	− 2.71±5.66	NS
20	128.0±8.2	134.8±5.2	+ 6.80± 8.99	NS	27.3±14.4	42.3±3.6	+15.00±9.15	NS
30	134.0±8.1	135.0±5.7	+ 1.00± 8.97	NS	29.0± 3.0	27.0±5.9	− 2.00±4.02	NS
40	133.7±9.6	135.5±4.6	+ 1.75±10.14	NS	21.3± 2.7	24.5±4.2	+ 3.25±2.83	NS
60	121.0±9.5	128.7±6.2	+ 7.75± 8.44	NS	15.1± 2.9	19.3±4.6	+ 3.00±4.46	NS
90	118.0±3.5	116.7±5.2	− 1.25± 1.78	NS	6.2± 1.4	12.5±1.6	+ 6.25±1.98	<0.05
120	105.5±8.4	108.0±5.6	+ 3.00± 2.87	NS	9.7± 1.7	17.3±4.7	+ 8.66±2.93	NS

[a] SEM

active transport of different amino acids in hamster small intestine [11], it must be concluded either that the determination of plasma levels of alpha-amino nitrogen is not a reliable measure of intestinal absorption of amino acids, or that in man the process of amino acid absorption is not affected by phenformin to the same degree as in *in vitro* studies. The increase in fasting levels of plasma alpha-amino nitrogen may evidence the reduction of the gluconeogenesis, and then the "normal" course of the alpha-amino nitrogen curve in plasma after phenformin subsequent to intraduodenal administration of amino acids could be interpreted as the result both of the reduced absorption and the reduced conversion of amino acids to glucose.

As others have also shown [15], in the present study the intraduodenal infusion of amino acids in diabetic patients caused a greater increase in insulinemia than intravenous infusion of single amino acid. Administration of phenformin inhibited or diminished the increase in insulinemia induced by intraduodenally infused amino acids, but did not modify the increase in insulinemia after intravenous infusion of L-arginine. In the interpretation of these results the different experimental conditions should be taken into account. Conceivably, this effect was not manifested because the increase in insulinemia after intravenous infusion of L-arginine was small. However, Boshell *et al.* [16] and more recently Luyckx *et al.* [17] also found no influence of phenformin on indirectly dependent on disorders of intestinal absorption of amino acids. A possible alternative mechanism might be a local effect of a high concentration of phenformin in the intestinal wall [18–20], inhibiting mitochondrial respiration [21] and decreasing ATP in the mucosal tissue [22], thus decreasing the biosynthesis of some protein, insulinotropic factors in the intestinal wall [23].

A direct inhibitory effect of phenformin on the beta cells [24] is less probable, since this should also occur in the case of arginine-stimulated insulin release.

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