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LACK OF INFLUENCE OF ARGININE PRELOADING ON THE INSULIN RESPONSE TO I.V. GLUCAGON IN CHILDREN AND ADOLESCENTS

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In a previous study [3], our group showed that glucose preloading induced an augmented insulin response to i.v. glucagon stimulation which was not related to the concomitant blood glucose level.

In order to ascertain whether this phenomenon is specific for glucose or can also be elicited by other substances known to stimulate insulin release, such as amino acids, a study was carried out to determine the influence of arginine on the B-cell response to i.v. glucagon.

MATERIALS AND METHODS

The tests were performed in 9 normal children (6 boys and 3 girls) who underwent evaluation of their growth at our Institute. The children were aged between 7 6/12 and 14 3/12 years with a mean age of 8 6/12 years. Their height corresponded to the mean for their age ± 1 SD. The tests were performed after obtaining informed consent from the parents.

The insulin response was studied during and after a 30-min i.v. infusion of arginine [4] (0.5 g/kg) as a 50% solution in saline, which was followed by one bolus i.v. glucagon (Lilly - 0.03 mg/kg) injection 90 min after starting the arginine infusion, when insulin concentrations were assumed to have returned to normal. Within an interval of one week, the patients underwent a separate i.v. glucagon *only* test, each

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patient serving as his own control. All patients were on a regular diet. The tests were performed after an overnight fast. Blood was collected through an indwelling needle at 0, 30, 60, 90, 92, 95, 100, 110 and 120 min into heparinized tubes. For the glucagon *only* test, samples were collected at 0, 2, 5, 10, 20 and 30 min. Blood for glucose assay was separated, the tubes centrifuged and the plasma stored at —20 °C until assayed.

Blood glucose was measured with a Technicon Autoanalyzer by ferricyanid colorimetry. Plasma insulin was assayed using a double antibody radioimmunoassay [2]. Statistical evaluations were performed by using Student's *t*-test.

RESULTS

Table 1 presents the individual plasma insulin responses during the combined arginine-glucagon test as compared to the glucagon only test. Fig. 1 illustrates the mean blood glucose and plasma insulin values for the whole group.

From the table it may be seen that arginine induced an increase of plasma insulin in 8 out of 9 children, the peak occurring in most instances 30 min after the start of the infusion. The administration of i.v. glucagon when plasma insulin had returned to basal values induced a plasma insulin rise which in 8 of 9 instances was greater than that induced by arginine infusion. Comparing the insulin response during the combined test to that observed during the i.v. glucagon test it is seen that the insulin peak evoked by glucagon in the combined test was higher in 4 instances (patients no. 1, 4, 8, 9), similar in 3 (patients no. 2, 6, 7) and lower in 2 (patients no. 3, 5). The mean insulin peak induced by i.v. glucagon administration following arginine was higher (101.77 μU/ml ± 20.47 SEM) than that induced by glucagon alone (85.95 μU/ml ± 11.52 SEM); this difference was not statistically significant.

patient	arginine infusion		i.v. glucagon after arginine preloading		i.v. glucagon	
	basal value	peak	basal value	peak	basal value	peak
1	4.9	78.3	16.4	200.0	9.4	74.1
2	5.7	18.1	4.7	28.0	7.5	30.7
3	7.0	78.3	8.8	51.4	12.4	80.4
4	8.6	16.1	10.7	77.9	10.8	57.1
5	9.2	18.5	2.3	40.2	7.0	91.9
6	10.1	18.3	13.6	69.6	6.1	69.3
7	12.0	71.5	8.7	144.6	10.1	139.9
8	27.2	79.1	15.5	141.3	29.9	132.1
9	35.4	14.3	19.2	163.0	17.5	98.1
mean	13.34	43.61	11.1	101.77	18.4	85.95
±SEM	±3.54	10.53	1.86	20.47	5.82	11.52

Tab. 1 - Comparison between basal and peak values of insulin (μU/ml) during stimulation with arginine, glucagon after arginine preloading and glucagon only, in each individual patient.

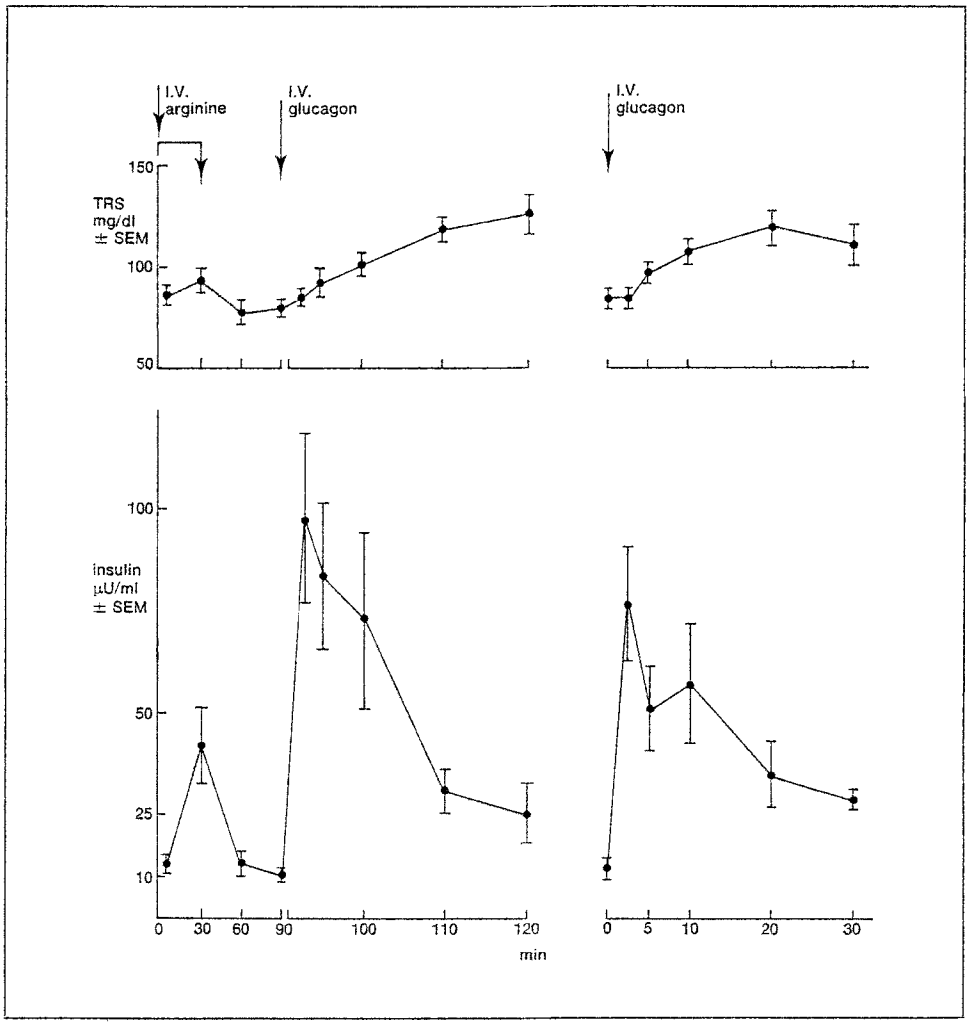

Fig. 1 - Blood glucose (mean ± SEM) and plasma insulin (mean ± SEM) response to arginine infusion and i.v. glucagon injected at 90 min compared to a separate i.v. glucagon test in 9 normal children. Blood glucose is expressed as total reducing substance (TRS) as measured with a Technicon Autoanalyzer by ferricyanid colorimetry.

Arginine induced little change in blood sugar (fig. 1), i.v. glucagon induced a slow rise of similar intensity in both tests.

The insulin peak occurred 30 min after starting the arginine infusion and between 2-5 min after administering glucagon. As seen from the figure there was no correlation between blood glucose level and insulin concentration.

DISCUSSION

Previous studies showed that glucose preloading increases the insulin response to glucagon by a mechanism which is growth hormone-dependent [5]. The present study

has shown that preloading with arginine does not cause a significant increase in response. On the basis of both direct measurement, *i.e.* the peak concentration, and indirect measurement, *i.e.* the response to i.v. glucagon, which allows us to estimate the readily-releasable pool of insulin, it would appear that arginine administered at the standard dose used (and possibly other amino acids as well) are weaker stimulants of insulin release than glucose. The increased insulin response to glucagon found after glucose preloading may possibly be due to specific sensitization of the glucagon receptor in the B-cell by glucose, or to a difference in intracellular action. It is well known that arginine acts on different receptors than glucose [7], a fact which may explain its lack of influence on the insulin response to glucagon.

Glucose promotes insulin release by acting on the receptor part of the B-cell adenyl cyclase thereby increasing the intracellular concentration of cyclic AMP [1,7]. It is not certain that glucagon, which is another stimulator of adenyl cyclase [6], acts on insulin release by an identical mechanism. Arginine seems to act on a stage subsequent to cyclic AMP formation [7]. This might account for the lack of additive effect of glucagon and arginine on insulin release.

SUMMARY

Nine normal children (6 males and 3 females) aged from 7 1/2 to 14 1/2 years underwent a 30-min arginine infusion (0.5 g/kg) followed at 90 min by one bolus i.v. glucagon injection (0.03 mg/kg). On a separate occasion the same children underwent an i.v. glucagon *only* test. No significant difference was found when the glucose and insulin responses in the two glucagon tests were compared, in contrast to previous findings that preloading with glucose resulted in a significantly increased response of insulin to glucagon.

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