INSULIN RESPONSE TO GLUCOSE AND INSULIN SENSITIVITY IN HYPOCALCAEMIC PATIENTS

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SYNOPSIS

Insulin response during intravenous glucose tolerance test (0.5 gm/kg body weight, infused over 3 minutes) was studied over a period of 90 minutes in eight hypoparathyroid patients in whom hypocalcaemia had not yet been adequately corrected with treatment (group A, mean serum calcium: 7.8 mg/100 ml) and seven hypoparathyroid patients in whom treatment had restored normocalcaemia (group B, mean serum calcium: 9.1 mg/100 ml). All the subjects except one were female, and the two groups were comparable in age and dosage of vitamin D_2 and calcium supplements. Both the acute and the delayed insulin response to glucose were impaired in group A whose mean "insulin area" was significantly lower ($p < 0.001$) than that of group B. There was, however, no difference in glucose tolerance between the two groups. Insulin: glucose ratio was consistently and significantly lower in the hypocalcaemic subjects. Insulin-glucose tolerance test performed in two hypocalcaemic and two normocalcaemic subjects revealed markedly enhanced sensitivity to exogenous insulin in the hypocalcaemic subjects. It is concluded that acute and delayed insulin response to glucose are diminished in hypocalcaemic patients and that this is associated with increased insulin sensitivity. Impaired secretion of hormones antagonistic to insulin action could have been responsible for the altered insulin sensitivity, as calcium may also play an important role in the secretion of these hormones.

INTRODUCTION

The importance of calcium in insulin secretion is well established in-vitro (Grodsky and Bennett, 1966; Milner and Hales, 1967; Curry et al., 1968; Trifaro, 1977). The influence of serum calcium on insulin release has been reported in animals (Littledike et al., 1968; Blum et al., 1973). In man, hyperparathyroidism increases and hypoparathyroidism decreases insulin response to oral glucose challenge (Yasuda et al., 1975). Insulin secretion in response to glucose is biphasic — an acute insulin response which peaks within a few minutes followed by delayed response (Porte Jr and

Bagadade, 1970). In this study, the acute as well as the delayed insulin response to glucose were studied in patients with hypocalcaemia. In some cases sensitivity to insulin was also assessed.

MATERIALS

Fifteen adult patients (14 females and 1 male) with hypoparathyroidism due to thyroid surgery were studied. All of them were on treatment with vitamin D_2 and calcium lactate. Seven of the patients had their serum calcium already restored to normal (mean 9.1 mg/d1; range: 8.7 — 10.1 mg/d1). They served as control for the rest of the patients who had not yet responded adequately to therapy and who were still hypocalcaemic (mean 7.8 mg/d1; range: 7.3 — 8.4 mg/d1). All patients were euthyroid, were not obese and were otherwise in good health. The two groups were comparable in age and dosage of medication (Table 1).

METHOD

The subjects took a high carbohydrate diet (about 3,000 cal) for at least three days prior to the test. On the day of the test, blood was drawn in the fasting state for the estimation of plasma glucose, insulin and calcium. Intravenous glucose (0.5 gm/kg body weight) was infused over 3 minutes and blood drawn for estimation of plasma glucose and insulin at 5 mins, 10 mins, 20 mins, 30 mins, 45 mins, 60 mins and 90 mins. Insulin "area", glucose "area", (area beneath the insulin curve and glucose curve respectively in the plot) and insulin; glucose ratios were calculated for each patient. The mean k values (measure of rate of fall of plasma glucose) of the groups were also calculated. On the second day, two hypocalcaemic and two normocalcaemic subjects, in addition, underwent a test of sensitivity to exogenous insulin by the method of Engel and Scott (1950). After an overnight fast, blood was obtained for basal glucose level. Soluble insulin (0.1 unit/kg body weight) was administered intravenously and blood glucose assessed at 30, 60, 90, 120, 150 and 180 minutes after insulin infusion. Glucose (0.8 gm per kg body weight) was given orally immediately after blood sampling for glucose at 30 minutes. Plasma insulin was estimated by radioimmunoassay using charcoal for separating free from bound hormone (Albano et al., 1972). Plasma glucose was measured by glucose oxidase method and plasma calcium by colorimetric method using the Technicon Autoanalyser.

RESULTS

As in Figure 1, plasma glucose values were not significantly different between the two groups of patients at the various points in time during the test. The mean glucose "area" of hypocalcaemic subjects, 15.83 ± 3.47 (SD) gm mins, is not significantly different from that of normocalcaemic subjects, 14.57 ± 2.62 gm mins. The mean k values of the two groups, 3.74 and 4.2 respectively were both within normal range (3.0 — 4.9). In contrast, as Figure 2 shows, plasma insulin level of hypocalcaemic subjects was significantly lower than that of normocalcaemic subjects at all points; the difference is seen even in the basal value. The mean insulin "area" of the hypocalcaemic subjects 2.51 ± 0.89 (SD) mU mins, is significantly lower ($p < 0.001$) than that of the normocalcaemic subjects, 5.74 ± 1.78 (SD) mU mins. This insulin response of the normocalcaemic subjects was normal by the criteria of Kipnis (1970). As expected, the derived insulin: glucose ratio at all points was significantly lower in the hypocalcaemic subjects (Figure 3). Response of plasma glucose to exogenous insulin is shown in Figure 4. The hypocalcaemic subjects had a more pronounced drop in plasma glucose (at 30 minutes) and a clearly slower return to basal level, compared to the normocalcaemic subjects. The response of the latter was normal by the criteria of Engel and Scott (1950).

TABLE 1

Details of Subjects Studied

Subjects	Age (yrs) (Mean and Range)	Calciferol Intake (mg) (Mean and Range)	Ca Lactate Intake (gm) (Mean and Range)
Hypocalcaemic (8)	27.3 (21 — 34)	6.9 (5 — 8.75)	4.2 (3.6 — 6)
Normocalcaemic (7)	26.4 (21 — 32)	7.3 (5 — 10)	4.6 (3.6 — 6)

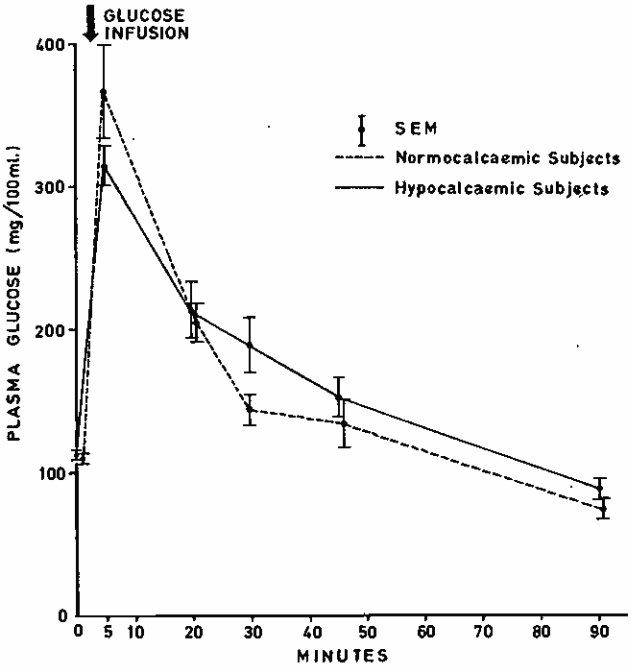

Fig. 1 Plasma glucose response to intravenous glucose in seven normocalcaemic and eight hypocalcaemic subjects.

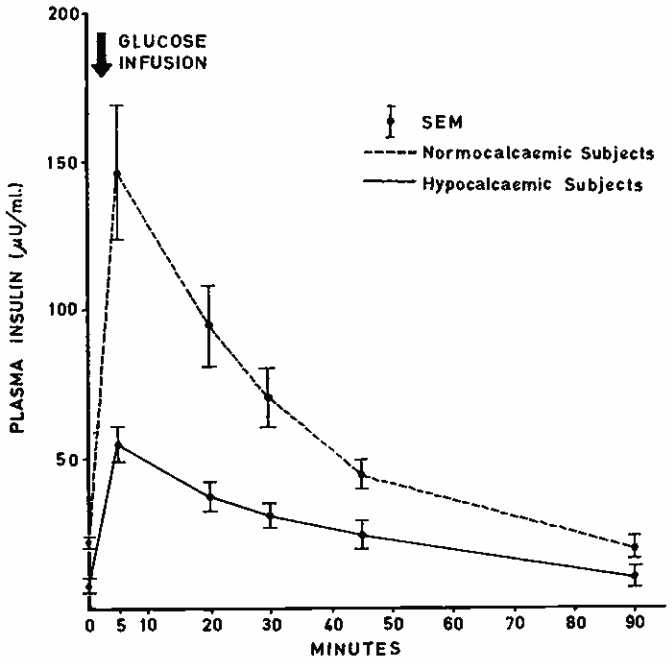

Fig. 2 Plasma insulin response to intravenous glucose in seven normocalcaemic and eight hypocalcaemic subjects.

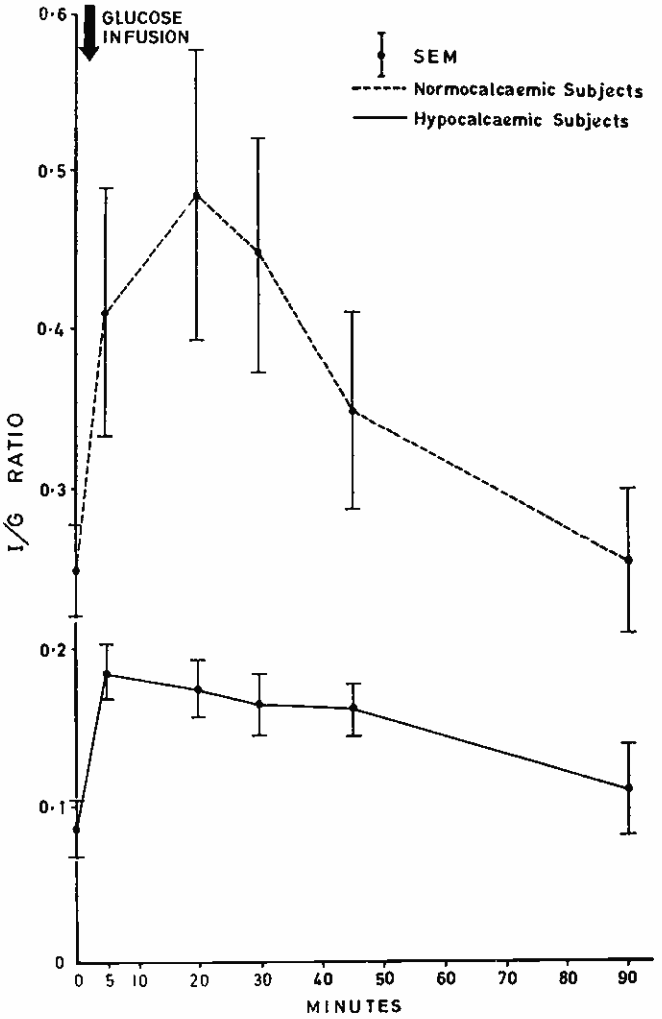

Fig. 3 Insulin: glucose (I/G) ratios following intravenous glucose in seven normocalcaemic and eight hypocalcaemic subjects.

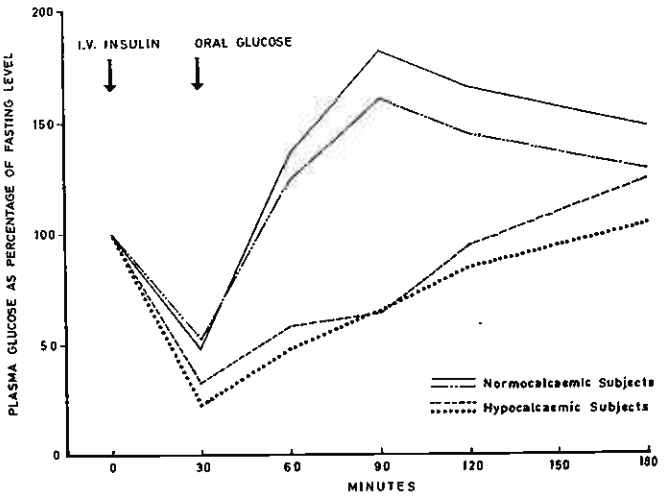

Fig. 4 Plasma glucose response to exogenous insulin in normocalcaemic and hypocalcaemic subjects.

DISCUSSION

This study shows that the acute insulin response to glucose challenge is diminished in hypocalcaemic hypoparathyroid patients. This was associated with a low basal insulin level. The delayed response is also diminished in these subjects as raised plasma glucose was sustained for at least 40 minutes and plasma insulin levels were consistently lower throughout.

In contrast, normocalcaemic hypoparathyroid subjects had normal insulin response. Since the two groups are comparable in age, sex and dosage of medication, it would seem that hypocalcaemia itself is responsible for the diminution in insulin response. In this regard it is interesting to note that hypocalcaemia of pseudohypoparathyroidism is also associated with diminished insulin response to oral glucose (Yasuda et al., 1970) supporting the hypothesis that it is hypocalcaemia and not the level of circulating parathyroid hormone that causes the difference in insulin response. In-vitro work (Kim et al., 1971) has also confirmed that parathyroid hormone does not itself influence insulin secretion.

The diminished insulin response of hypocalcaemic subjects is associated with normal glucose tolerance whereas normocalcaemic subjects have normal glucose tolerance associated with normal insulin response. The implication therefore is that the hypocalcaemic subjects are abnormally sensitive to insulin, and are thus able to maintain normal glucose tolerance with a smaller output of insulin in response to glucose. This was confirmed in two hypocalcaemic hypoparathyroid subjects in whom exogenous insulin (0.1 unit/kg body weight) induced severe hypoglycaemia and glucose administered at the time of hypoglycaemia brought about only a sluggish return to the basal level plasma glucose. For these reasons this test was not carried out in the other hypocalcaemic patients.

The reason for enhanced peripheral sensitivity to insulin remains to be determined. It could be a direct effect of hypocalcaemia itself on the tissues, perhaps by increasing receptor binding of insulin. Alternatively hypocalcaemia could cause a simultaneous reduction in secretion of those hormones which antagonise the action of insulin, such as glucagon, catecholamines, cortisol and growth hormone. Calcium is required in exocytosis, the energy-dependent process by which certain hormones are released (Trifaro, 1977) and there is good evidence of exocytosis in the adrenal medulla, (De Robertis and Vaz Ferreira, 1959) in the α and β cells of the pancreas (Gomez et al., 1968; Lacey, 1961) and in the corticotroph (Rennels and Shinno, 1968), and somatotroph (Farquhar, 1961) of the pituitary gland.

Clinically however, none of the hypocalcaemic subjects had evidence of cortisol deficiency. It would be of great interest to study the secretion of these antagonistic hormones, in particular glucagon and catecholamines (in response to challenge) in the presence of hypocalcaemia.

Increased tissue sensitivity to insulin, whatever the mechanism mediating it, could contribute to reduced insulin secretion by causing hypoglycaemia which alters the set of β cells. None of the hypocalcaemia subjects had clinical hypoglycaemia. However there is good in-vitro evidence that calcium directly influences secretion of insulin and that over the range of calcium concentration of 0.5 — 4 mEq/L (ionic calcium) insulin release in response to glucose in proportional to calcium concentration of the perfusate (Curry et al., 1968).

The normal glucose tolerance in hypocalcaemic subjects would suggest that decreased insulin secretion and increased tissue response to insulin are exactly balanced. This could have been achieved by the modulation of insulin secretion (in response to increased tissue sensitivity to insulin) in the background of diminished insulin secretion directly due to hypocalcaemia. The absence of clinical hypoglycaemia could thus be explained.

REFERENCES

1. Albano, J.D.M., Ekins, R.P., Maritz, G., and Turner, R.C.: A sensitive, precise radioimmunoassay of insulin relying on charcoal separation of bound and free moieties. Acta. Endocrinol. (Copenhagen) 70 : 487-509, 1972.
2. Blum, B.J., Wilson, R.B. and Kronfeld, D.S.: Prapartuale Hyperinsulinamie and kalziumabhangige insulinsekretion bei der kuh. Schweiz Med Wochenschr 103 : 849-852, 1973.
3. Curry, D.L., Benett, L.L. and Grodsky, G.M.: Requirement for calcium ion in insulin secretion by the perfused rat pancreas. Am. J. Physiol. 214: 174-178, 1968.
4. De Robertis, E. and Vaz Ferreira, A.: Electron microscope study of the secretion of catechol-containing droplets in the adrenal medulla. Exp. Cell Res. 12:568-574, 1957.
5. Engel, F.L. and Scott, J.L.: The insulin glucose tolerance test. A modified procedure for the detection of hypoglycaemic unresponsiveness in pituitary and adrenal insufficiency. J. Clin. Invest. 29:151-160, 1950.
6. Farquhar, M.G.: Origin and fate of secretory granules in cells of the anterior pituitary gland. Trans. N.Y. Acad. Sci. 23:346-351, 1961.
7. Gomez-Acebo, J., Parrilla, R. and Candela, L.R.: Fine structure of the A and D cells of the rabbit endocrine pancreas invivo and incubated in-vitro. I. Mechanism of secretion of the A cells. J. Cell Biol, 36 : 33-44, 1968.
8. Grodsky, G.M. and Benett, L.L.: Cation requirement for insulin secretion in the isolated perfused pancreas. Diabetes 15: 910-913, 1966.
9. Kim, H., Kalkhoff, R.K., Costrini, N.V., Cerletty, J.M. and Jacobson, M.: Plasma insulin disturbances in primary hyperparathyroidism. J. Clin. Invest. 50: 2596-2605, 1971.
10. Kipnis, D.M.: Insulin secretion in normal and diabetic individuals. Adv. Int. Med. 16:103-134, 1970.

11. Lacey, P.E.: Electron microscopy of the beta cell of the pancreas. Am. J. Med. 31: 851-859, 1961.
12. Littledike, E.T., Witzel, D.A. and Whipp. S.C.: Insulin: evidence for inhibition of release in spontaneous hypocalcaema. Proc. Soc. Exp. Biol. Med. 129: 135-139, 1968.
13. Milner, R.D.G. and Hales, C.N.: The role of calcium and magnesium in insulin secretion from rabbit pancreas studied in vitro. Diabetologia 3:47-49, 1967.
14. Porte Jr., D. and Bagadade, J.D.: Human insulin secretion: an integrated approach. Ann. Rev. Med. 21:219-240, 1970.
15. Rennels, E.G. and Shiino, M.: Ultrastructural manifestations of pituitary release of ACTH in the rat. Arch. Anat. Histol. Embryol. 51: 575-590, 1968.
16. Trifaro, J.M.: Common mechanisms of hormone secretion. Ann. Rev. Pharmacol. Toxicol. 17: 27-47, 1977.
17. Yasuda, K., Hurukawa, Y., Okuyama, M. Kikuchi, M. and Yoshinaga, K. : Glucose tolerance and insulin secretion in patients with parathyroid disorders. Effect of ,serum calcium on insulin release. New Engl. J. Med. 292: 501-504, 1975.