RAPID COMMUNICATION

Insulin Secretion after Oral Calcium Load

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Synopsis

Oral glucose tolerance test with 50 g glucose was carried out with and without simultaneous oral administration of 3 g calcium lactate. Blood sugar and serum immunoreactive insulin (IRI) were measured before and 30, 60, 90 and 120 min after glucose load in 11 diabetics and 11 non-diabetics. Similar oral glucose tolerance test was performed with and without intravenous administration of 10 ml 8.5% calcium gluconate in 6 diabetics and 6 non-diabetics. Maximum serum level after glucose load (Max IRI), maximum rise of serum IRI following glucose load (Max Δ IRI), maximum Δ IRI/ Δ BS following oral glucose load (Max Δ IRI/ Δ BS), the area surrounded by the Δ IRI curve and the abscissa (Σ Δ IRI) and the area surrounded by the Δ IRI/ Δ BS curve and the abscissa (Σ Δ IRI/ Δ BS) significantly increased in diabetics but not in non-diabetics after oral calcium load, whereas intravenous calcium load failed to influence any of these values. Oral calcium load thus appears to augment glucose-induced insulin secretion only in diabetics, suggesting some abnormality in the mechanism of insulin secretion with reference to calcium in diabetes mellitus.

The important role of calcium in cell activities especially hormone secretion has recently been widely recognized and numerous hormones were shown to require calcium in their secretion. The secretion of insulin has also been shown to be intimately connected with calcium (Hales *et al.*, 1968) and the physiological role of calcium on the insulin secretory mechanism has been clarified. In order to elucidate the role of calcium as one of the insulin secretagogues at the clinical level, oral and intravenous calcium load was given prior to oral glucose tolerance test in diabetics and non-diabetics, to study the effect of calcium load on glucose-induced insulin secretion

in an attempt to have a better understanding of the mechanism of disturbance of insulin secretion in diabetes mellitus.

Materials and Methods

Seventeen patients with diabetes mellitus between 31 and 76 years of age and 17 patients without diabetes mellitus between 18 and 75 years of age were studied. Oral load of 50 g glucose was given with and without oral load of 3 g calcium lactate immediately after the glucose load in 11 diabetics and 11 non-diabetics and similar load with and without intravenous injection of 10 ml 8.5% calcium gluconate was given in 6 diabetics and 6 non-diabetics. Blood sugar was measured by Somogyi-Nelson method and serum IRI by radioimmunoassay of double antibody method before and 30, 60, 90 and 120 min after glucose load. Maximum blood sugar level (Max BS), maximum serum IRI (Max IRI), maximum rise of blood sugar level from the fasting state (Max Δ BS), maximum rise of serum IRI from the fasting level (Max Δ IRI), maximum Δ IRI/ Δ BS (Max Δ IRI/ Δ BS), cumulative rise of blood

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sugar calculated as the area surrounded by the BS curve and the abscissa expressed as $BS_{80} + BS_{60} + BS_{90} + 1/2 BS_{120}$ ($\Sigma \Delta BS$) and cumulative rise of serum IRI calculated as the area surrounded by the IRI curve and the abscissa expressed as $IRI_{30} + IRI_{60} + IRI_{90} + 1/2 IRI_{120}$ ($\Sigma \Delta IRI$) were obtained as the indices for insulin secretion and glucose metabolism after oral glucose loading. Blood sugar was determined by Somogyi-Nelson's method and IRI by double antibody method. Statistical analysis was conducted by Student's *t* test and Wilcoxon's match-pairs sign-ranks test.

Results

As shown in Tables 1 and 2, maximum IRI after glucose load significantly rose after simultaneous oral calcium load in 11 diabetics ($P < 0.05$) above the level without calcium in paired observation, whereas the comparison of absolute values reveals significantly lower Max ΔBS in the group with oral calcium load. Similarly, Max ΔIRI ($P < 0.05$), maximum $\Delta IRI/\Delta BS$ ($P < 0.05$), $\Sigma \Delta IRI$ ($P < 0.05$) and $\Sigma \Delta IRI/\Sigma \Delta BS$ ($P < 0.05$) were also significantly higher after oral calcium load than without it.

None of these values showed significant

changes after oral calcium load in 11 non-diabetics. None of these values showed significant changes after intravenous calcium injection in both diabetics and non-diabetics.

Max BS, and $\Sigma \Delta BS$ did not change significantly after oral or intravenous calcium load in diabetics and non-diabetics.

Discussion

Since glucose is the best stimulant for insulin secretion, the rise of serum IRI following glucose load has been used for the evaluation of insulin secretion. Especially since insulinogenic index or $\Delta IRI/\Delta BS$ was devised as an index of insulin secretion (Seltzer and Smith, 1959), the insulin secretion in response to glucose load was expressed more quantitatively. In the present study, oral calcium load definitely increased insulin secretion in response to glucose load, according to maximum serum IRI (Max IRI), maximum rise of serum IRI from the fasting level (Max ΔIRI), maximum $\Delta IRI/\Delta BS$, the cumulative serum insulin rise ($\Sigma \Delta IRI$) and cumulative serum insulin rise

Table 1. Oral Glucose tolerance test with (CaOGTT) and without (OGTT) oral calcium load in diabetics. Maximum levels and rises of blood sugar (BS) and IRI. Differences between OGTT and CaOGTT was tested by student's *t* test and OGTT-CaOGTT by Wilcoxon's match-pairs sign-ranks test. BS is expressed in mg/dl and IRI in $\mu U/ml$.

Case	Age	Sex	OGTT				CaOGTT				OGTT-CaOGTT			
			Max BS	Max IRI	Max ΔBS	Max ΔIRI	Max BS	Max IRI	Max ΔBS	Max ΔIRI	Max BS	Max IRI	Max ΔBS	Max ΔIRI
1	57	F	244	11.9	132	3.9	172	15.2	78	7.4	72	- 3.3	54	- 3.5
2	43	M	260	33.0	154	12.7	336	50.9	92	16.2	-76	-17.9	62	- 4.5
3	72	M	216	26.8	123	20.2	225	40.2	117	31.9	- 9	-13.4	6	-11.7
4	35	M	204	16.2	92	7.7	216	13.0	104	5.2	-12	3.2	-12	2.5
5	75	M	350	26.0	163	2.0	271	32.0	95	4.0	79	- 6.0	68	- 2.0
6	45	M	285	8.9	128	2.1	244	9.8	142	3.0	41	- 0.9	-14	- 0.9
7	75	M	248	28.4	126	19.1	243	44.6	126	31.1	5	-16.2	0	-12.0
8	76	F	450	4.8	167	0.9	485	11.2	173	4.6	-35	- 6.4	- 6	- 3.7
9	35	M	435	10.4	166	0	344	8.9	102	3.3	91	1.5	64	- 3.3
10	53	M	357	7.3	146	0.6	299	10.8	107	3.7	58	- 3.5	39	- 3.1
11	31	F	322	22.0	106	9.0	268	39.4	128	25.7	54	-17.4	-22	-16.7
AV $\pm$ SEM			306 ± 25	17.8 ± 2.9	136 ± 7	7.1 ± 2.1	282 ± 25	25.1 ± 4.8	114 ± 8	12.3 ± 3.4	25 ± 16	- 7.3 ± 2.3	21 ± 10	- 5.3 ± 1.7
Significance							ns	ns	$P < 0.05$	ns	ns	$P < 0.05$	ns	$P < 0.05$

Table 2. Oral glucose tolerance test with (CaOGTT) and without (OGTT) oral calcium load in diabetics. Ratio between Δ IRI and Δ BS, integrated values and the ratio of integrated values. Differences between OGTT and CaOGTT was tested by student's *t* test, and OGTT-CaOGTT by Wilcoxon's match-pairs sign-ranks test. BS and IRI were expressed as in Table 1.

Case	Age	Sex	OGTT				CaOGTT				OGTT-CaOGTT			
			Max Δ IRI /BS	$\Sigma \Delta$ BS	$\Sigma \Delta$ IRI	$\Sigma \Delta$ IRI /BS	Max Δ IRI /BS	$\Sigma \Delta$ BS	$\Sigma \Delta$ IRI	$\Sigma \Delta$ IRI /BS	Max Δ IRI /BS	$\Sigma \Delta$ BS	$\Sigma \Delta$ IRI	$\Sigma \Delta$ IRI /BS
1	57	F	0.051	375	9.0	0.025	0.147	168	13.7	0.082	-0.096	267	-4.7	-0.057
2	43	M	0.082	375	20.3	0.054	0.338	198	42.5	0.215	-0.299	177	-22.2	-0.057
3	72	M	0.336	305	37.7	0.124	0.326	258	72.1	0.279	0.010	47	-34.4	-0.155
4	35	M	0.158	282	24.0	0.085	0.450	315	18.0	0.057	-0.292	-33	6.0	0.028
5	75	M	0.012	292	1.0	0.003	0.064	234	13.0	0.056	-0.052	58	-12.0	-0.053
6	45	M	0.030	274	6.4	0.023	0.044	452	9.0	0.020	-0.014	-178	-2.6	0.003
7	75	M	0.152	312	26.2	0.084	0.247	240	30.3	0.126	-0.095	72	-4.0	-0.042
8	76	F	0.007	559	2.5	0.004	0.027	547	14.0	0.026	-0.020	12	-11.5	-0.022
9	35	M	0	452	0	0	0.118	287	28.0	0.098	-0.118	165	-28.0	-0.098
10	53	M	0.004	338	0.8	0.002	0.043	316	3.9	0.012	-0.039	22	-3.1	-0.010
11	31	F	0.080	324	16.6	0.051	0.210	370	67.2	0.181	-0.130	-46	-50.6	-0.130
AV $\pm$ SEM			0.083 $\pm$ 0.031		13.1 $\pm$ 3.7	0.041 $\pm$ 0.012	0.183 $\pm$ 0.043	307 $\pm$ 34	28.3 $\pm$ 7.1	0.104 $\pm$ 0.029	-0.100 $\pm$ 0.028	60 $\pm$ 34	-15.1 $\pm$ 5.1	-0.063 $\pm$ 0.019
Significance			ns	ns	ns	ns	ns	ns	ns	ns	P<0.05	ns	P<0.05	P<0.05

divided by cumulative blood sugar rise ($\Sigma \Delta \text{IRI} / \Sigma \Delta \text{BS}$) in diabetics but not in non-diabetics. Intravenous calcium load had such effect neither in diabetics nor in non-diabetics, possibly due to a shorter duration of hypercalcemia.

Calcium has been known as one of the many insulin secretagogues (Brisson *et al.*, 1972). Insulin secretion stimulated by glucose was abolished by removal of calcium from the incubation medium and calcium apparently acts on the cell membrane or enter the cell before insulin release (Malaisse, 1973). Microtubules apparently play an important role in the insulin release (Malaisse, 1973). Extracellular calcium is required for the release of insulin. Rate of insulin release by isolated islets has been correlated with the rate of ^{45}Ca uptake (Curry, 1968; Grodsky, 1966). Calcium accumulation in the cells would remove an electrostatic energy barring the granule-cell interval. Calcium thus possibly triggers insulin release by causing activation or some biochemical damage in the configuration of cell microtubular-microfilamentous system. Calcium antagonists, on the other hand, inhibit insulin secretion (Davis *et al.*, 1975; Malaisse *et al.*, 1976).

The relationship between serum calcium and insulin secretion has also been studied. Salvesen, as far back as in 1954, reported a decrease of glucose tolerance in dogs with hypocalcemia induced by parathyroidectomy (Salvesen, 1954). On the other hand, insulin hyperresponsiveness was found in primary hyperparathyroidism with hypercalcemia (Kim *et al.*, 1971). Hypocalcemia was associated with a markedly reduced insulin secretory response and normal tissue insulin sensitivity, while hypercalcemia was associated with a normal insulin response but reduced tissue sensitivity (Amend *et al.*, 1975). Acutely induced hypocalcemia suppressed insulin secretion, while an intravenous calcium load in conjunction with oral glucose load decreased blood glucose

levels without a significant change in serum IRI (Gerö *et al.*, 1976). In patients with islet cell tumor, intravenous calcium infusion caused a release of insulin and proinsulin, but no such effect was obtained in normal subjects (Gaeke *et al.*, 1975).

The reason for the stimulation of insulin secretion only by oral calcium load and not by intravenous calcium load in diabetics remains unknown at present, especially since serum calcium probably rises much higher in the latter than in the former. Participation of gastrointestinal hormones as insulin secretagogue secreted in response to oral calcium load but not to intravenous calcium might explain such difference. The more pronounced insulin release in response to oral glucose load in diabetics than in non-diabetics might suggest some disturbance in insulin release in diabetics which is at least partially corrected by oral calcium load.

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