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SUPPRESSION OF INSULIN RELEASE BY CALCIUM ANTAGONIST IN HUMAN INSULINOMA IN VIVO AND IN VITRO
Its Possible Role for Clinical Use

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INDEXING WORDS

insulinoma; insulin; calcium-antagonist; hypoglycemia

SYNOPSIS

Intravenous infusion of diltiazem hydrochloride, calcium-antagonist, was performed on a 46-year-old woman with leucine-sensitive insulinoma which was beta cell adenoma histologically. It prevented the leucine-induced insulin secretion and hypoglycemic attacks effectively, although hypoglycemia persisted. Besides, oral administration of this agent lessened the hypoglycemic attacks. The inhibition of insulin release by the compound was demonstrated also in in vitro studies of incubation using the tumor tissues excised. These observations suggest the view that calcium-antagonist would be a useful agent to prevent the hypersecretion of hormones induced by functioning endocrine tumors.

INTRODUCTION

The presence of adequate extracellular concentration of calcium has been recognized to be essential for the secretory process of hormones to a variety of stimuli and the insulin secretion has been demonstrated to be related to the calcium concentration in vivo and in vitro.[4,5,6,15)]

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As for insulin release, it is known to be associated with the calcium uptake into the secretory cells.[9,11] Recently there have been several reports that the inhibition of the entry of calcium ions into the cell and/or mobilization of the intracellular calcium by calcium antagonist reduces hormone secretion.[6,13] Furthermore, insulin release is revealed to be parallel to extracellular calcium concentration in its certain range.[6,15]

Calcium-antagonist, therefore, was applied to a patient with insulinoma to study whether it prevents the insulin release in vivo and also in vitro using the excised insulinoma tissues.

MATERIALS AND METHODS

A. In Vivo Studies.

Case summary.

A 46-year-old Japanese housewife was in good health until 11 months before admission to our hospital, when she had recurring episodes of nausea, tremor, sweating and occasional syncopal attacks with the duration of about thirty minutes and recovery by bed rest or meals. These episodes tended to occur before meals or after its avoidance. The attacks occurred almost monthly and no special measures were taken before her admission.

Physical examination revealed no abnormalities except progressive obesity beginning around the onset of her symptoms. Her height was 148 cm, whereas her weight 70 kg.

Fasting hypoglycemia (blood glucose 20-80 mg/100 ml) with elevated plasma insulin (36-151 μU/ml) was repeatedly observed even after her hospitalization.

Oral glucose tolerance test (50 g), tolbutamide infusion test (1 g), glucagon infusion test (1 mg) and l-leucine loading test (150 mg/kg orally) showed abnormal insulin responses and hypoglycemia (Table 1).

Oral glucose tolerance test showed only a small rise of glucose, while insulin level was raised to high levels of about 150 μU/ml. After the operation as shown in parentheses, a tiny elevation of blood glucose and normal value of insulin were noted. Intravenous administration of 1 g tolbutamide and 1 mg glucagon also showed hyperresponse of insulin release. Leucine loading test caused a marked decrease of blood glucose, and the patient

complained of palpitation, sweating 30 minutes after its initiation. The test was obliged to stop at 60th minute when blood glucose showed only 9 mg/100 ml and the patient fell into unconsciousness (Table 1).

Table 1 Preoperative diagnostic findings.

Time (min.)		Blood Glucose (mg/100ml)		Plasma Insulin (μ units/ml)	
			Post ope.		Post ope.
OGTT	0	33	(110)	50	(12)
(50 g)	30	36	(184)	82	(40)
	60	68	(192)	149	(55)
	120	62	(194)	151	(59)
	180	24	(114)	118	(19)
Tolbutamide Test (1 g, i.v.)					
	0	81		85	
	3	73		150	
	10	65		215	
	30	36		123	
	60	42		126	
	120	39		132	
Glucagon Test (1 mg, i.v.)					
	0	20		44	
	5	61		51	
	10	79		48	
	20	104		102	
	30	111		77	
	60	92		67	
	120	43		68	

Leucine Loading Test (150 mg/kg orally)

Time (min.)	0	5	10	20	30	45	60	
Blood Glucose (mg/100ml)	31	30	29	23	18	12	9	stopped !

Superior mesenteric angiography and endoscopic retrograde cholecysto-pancreaticography revealed neither hypervascularities nor space occupying lesions. Otherwise the other laboratory findings were within normal range. At the exploratory laparotomy a solitary tumor was found in the head of pancreas. Histological examination revealed the benign B-cell adenoma.

Insulin extracted with acid ethanol from the tumor and a portion of the pancreatic body indicated 5.06 units and 1.45 units/g wet tissue, respectively. Plasma insulin was examined every 30 minutes during the operation. Forty μU/ml of insulin fell to 9 μU/ml as shortly as 3 minutes after the resection of the insulinoma.

Studies using calcium-antagonist.

Intravenous infusion of calcium-antagonist (Diltiazem hydrochloride,[12] Tanabe Seiyaku Co., Ltd., Osaka, Japan) was done at bed rest after overnight fast. Polyethylene catheters were set into both cubital veins for the infusion of diltiazem hydrochloride at one side and the blood sampling at the other side; both of them were kept patent by continuous dripping of saline.

Diltiazem hydrochloride, 44 mg dissolved in 250 ml of saline, was infused for two hours at the same rate by a peristaltic pump (Type MP-101, Tokyo Rikakikai Co., Tokyo, Japan). Twenty minutes after beginning of the infusion of calcium-antagonist l-leucine (150 mg/kg body weight) was administered orally. Furthermore, to verify the prevention from the hypoglycemic attack, the oral administration of diltiazem hydrochloride (180 mg/day) to the patient with insulinoma for 14 days was tried.

B. In Vitro Studies.

Islets of Langerhans from adult male Wistar rats weighing 200-250 g were isolated by the method of Lacy and Kostianovsky.[8] Five islets were preincubated in 500 μl of Krebs-Henseleit bicarbonate buffer, pH=7.4, (KHBB) containing 3.3 mM glucose and 0.5% bovine serum albumin for 20 minutes under gas phase of 95% O_2 - 5% CO_2 at 37°C, subsequently transferred to the medium (KHBB) containing 8.3 mM glucose and various concentration of diltiazem hydrochloride ranging from 5-200 μM, and incubated for 60 minutes, otherwise under the same conditions as in the preincubation. Insulin released into the medium at the last incubation was measured.

On the other hand sliced tissues (mean wet weight was 7.6 mg) of an insulinoma excised from this patient were preincubated in 3 ml of KHBB containing 3.3 mM glucose and 0.5% bovine serum albumin for 30 minutes under gas phase of 95% O_2 - 5% CO_2 at 37°C. Thereafter tissues were incubated successively in KHBB containing 3.3 mM glucose, 3.3 mM glucose plus 10 mM l-leucine and 3.3 mM glucose plus 10 mM l-leucine plus 20 μM diltiazem hydrochloride for 30 minutes in order after the preincubation. As for the control, sliced normal pancreatic tissues excised with insulinoma were incubated in KHBB containing 3.3 mM glucose in the same way. Insulin was measured in each medium.

C. Analytical Method.

Insulin was measured by radioimmunoassay[10] using porcine and rat insulin (Lilly Research Laboratories, U.S.A.) as the standard and blood glucose was determined by autoanalyzer method. The insulin antibody was kindly supplied by Dr. Y. Kohga (Shimizu Seiyaku Kabushikigaisha, Shimizu, Japan).

Bovine serum albumin and collagenase were purchased from Armour Pharmaceutical Co., U.S.A. and Boehringer Manheim Co., West Germany, respectively. Other chemical reagents for in vitro studies were obtained from Nakarai Chemical Co., Kyoto, Japan.

For the statistical analysis Student's t-test was used.

RESULTS

1. Studies Using Calcium-Antagonist.

Plasma insulin changed from 66 μU/ml before to 53 μU/ml five minutes after the initiation of the intravenous infusion of diltiazem hydrochloride, and then increased to 87 μU/ml, but in spite of the loading of l-leucine further elevation of insulin was not detected during the infusion period of this compound. Besides, plasma insulin level was elevated shortly after the two hours' infusion (Fig. 1).

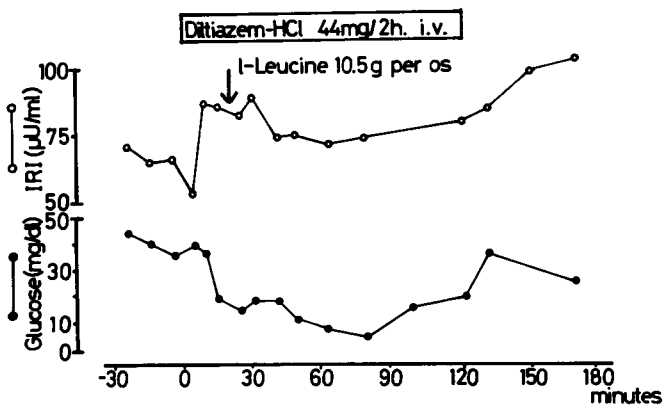

Fig. 1 Plasma insulin (IRI) and blood glucose levels in a patient with B-cell adenoma of pancreas. No increment of insulin was observed after oral leucine loading in the presence of diltiazem hydrochloride, whereas its elevation was noted after the discontinuance of infusion.

Plasma glucagon was also measured in the course of the investigation using specific antiserum to pancreatic glucagon (purchased from Dr. Unger). Mean plasma glucagon level before the infusion of the compound was 125 pg/ml, and after its initiation no remarkable change of glucagon was noted even by the loading of l-leucine in spite of the presence of severe hypoglycemia. However, after its discontinuance, plasma glucagon increased promptly to 183 pg/ml and such high level continued for thirty minutes, followed by its decrease to 100 pg/ml. Details on the glucagon response have been reported elsewhere.[14)]

Meanwhile, blood glucose prior to the leucine loading continued to decrease to as low as 5 mg/100 ml at 65th minute. In spite of the presence of such a severe low blood glucose level hypoglycemic symptoms were not observed, although it occurred at the previous test by l-leucine in the absence of calcium-antagonist. The levels of blood glucose were not different largely from its values observed at the former leucine loading test. As soon as the intravenous infusion of diltiazem hydrochloride was completed, rapid rise of blood glucose was noted.

Plasma sodium, potassium, chloride, calcium and phosphate which were checked repeatedly through the test were within normal ranges and no remarkable change was observed. Similarly, no detectable changes of blood pressure and pulse rate were noticed.

Oral administration of the compound in divided doses of 180 mg a day for 14 days reduced the frequency of hypoglycemic attacks contrasted with the control period of no medication and no remarkable side effects were noted.

2. In Vitro Studies.

Diltiazem hydrochloride suppressed 8.3 mM glucose-induced insulin release from isolated rat islets dose-dependently: the control value was 5.60 ± 0.88 ng/islet/60 min. (n=5), while at the presence of 10, 20 and 100 μM diltiazem hydrochloride, the value was 2.91 ± 0.22 (n=5), 2.30 ± 0.25 (n=5) and 1.54 ± 0.35 ng/islet/60 min. (n=3) respectively (Fig. 2).

Insulin release from sliced insulinoma tissues of this patient at the first incubation after 30 minutes' preincubation was shown as 100% and the actual value was 2.74 ± 0.18 and 2.70 ± 0.00 ng/mg wet tissue weight in the control and experimental group, respectively. At the second and third incubation, insulin

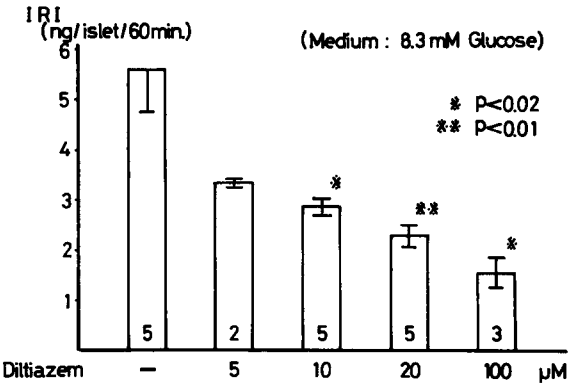

Fig. 2 Effect of various concentration of diltiazem hydrochloride on 8.3 mM glucose-induced insulin release from isolated rat islets. After 20 minutes' preincubation, five islets from male Wistar rats were incubated in 500 μl of KHBB containing 8.3 mM glucose and various concentration of diltiazem hydrochloride for 60 minutes. Insulin in the incubation medium was radioimmunoassayed using rat insulin as standard. Number of observations is shown at the lower part of each bar.

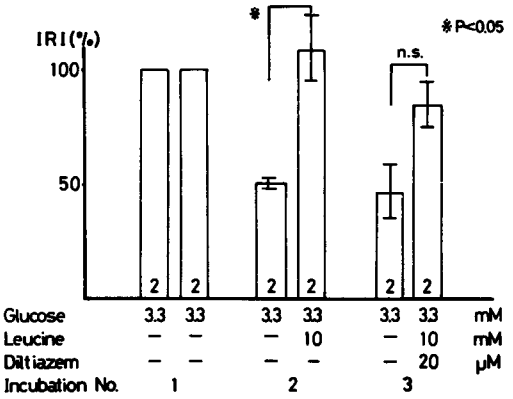

Fig. 3 Effect of diltiazem hydrochloride on leucine-induced insulin release from sliced human insulinoma tissues. In experimental group, the right hand column of each set of two columns, sliced human insulinoma tissues were incubated in 3.3 mM glucose, 3.3 mM glucose plus 10 mM l-leucine and finally 20 μM of diltiazem hydrochloride in addition each for 30 minutes, while those incubated in mere 3.3 mM glucose for 30 minutes successively three times in control groups are shown at the left hand column of each set of two columns. Insulin values at the first incubations are shown as 100% in both control and experimental groups. Number of observations is written at the lower part of each bar. Note the inhibitory effect of diltiazem hydrochloride on leucine-induced insulin release.

values were 50.9% and 47.2% in the former group, respectively, while in the latter group it was 109.7% and 84.7%, respectively (Fig. 3). Increment of insulin release by l-leucine compared with the control, 213%, shown at the second incubation was reduced to 177% by the compound, indicated at the third incubation.

DISCUSSION

Although many trials have been reported, there has been no satisfactory non-surgical method for the prevention of the hypoglycemia in insulinoma. For example, diazoxide or streptozotocin can reduce the hyperinsulinemia non-surgically but these drugs are toxic and induce severe hypoglycemic attacks.[1,3)]

Recently Blum and co-worker observed that propranolol, beta-adrenergic blocker, had effectively prevented the recurrent hypoglycemic attacks of the patient suffering from benign beta cell adenoma without any side effects till the surgery.[2)] Similarly, in the present case, propranolol corrected the basal hyperinsulinemia as well as the increased insulin secretion by glucose or arginine.

But the drug is generally considered not to be favored in the patients with bradycardia or asthma. Therefore, we studied the effectiveness of calcium-antagonist in insulinoma as it is useful for cardiac illness and it is thought to induce the inhibition of calcium entry into cells and/or intracellular calcium mobilization.[7)] Besides, calcium influx into the beta cell of Langerhans occurs with glucose-induced insulin release simultaneously.

As the patient was strikingly sensitive to leucine, leucine loading test was chosen to observe the efficiency of diltiazem hydrochloride.

The drug suppressed the further secretion of insulin by l-leucine and after the end of its infusion gradual enhancement of insulin secretion was observed. Serum glucagon did not change much in spite of the presence of low glucose level during the presence of diltiazem hydrochloride, but caused rapid increase after its discontinuance.

These observations suggest that the compound suppressed both insulin and glucagon release, and that the hypoglycemic state might be induced by the high ratio of insulin to glucose level. The

compound was confirmed to inhibit the insulin release dose-dependently in vitro as well as verapamil, another agent of calcium-antagonist. The studies using tumor tissues also indicate the direct suppression of insulin secretion from the tumor tissues. The reason of the rise of insulin values 5 minutes after the infusion of the drug is not certain but it would be due to the dilatation of the pancreatic vessels.

In the course of this test hypoglycemic symptoms, such as sweating, palpitation and unconsciousness, which were observed at previous study of leucine loading, were not noted despite the presence of as low glucose concentration as 5 mg/100 ml.

To our knowledge there have never been any reports on this kind of phenomenon. But the mechanism of the absence of hypoglycemic symptoms under the presence of calcium-antagonist has not been clarified. It might be due to the direct action of this agent on the central nervous system or the autonomic nervous system. It might be suggested in this case that calcium ion mobilization in the cell or into the cell of these tissues would be associated with the autonomic function.

The sensitivity of insulin or glucagon release to calcium-antagonist might vary with insulinomas. Therefore, further investigations are necessary to delineate the effect of calcium-antagonist on insulin-producing tumors.

The present observation in a single case of insulinoma, however, suggests the view that calcium-antagonist would be a useful agent to prevent the hypersecretion of hormones induced by functioning endocrine tumors. But care must be taken of the disorders induced by hypoglycemia when the drug was applied to insulinoma, because of the paucity of the symptoms.

REFERENCES

1. Bailey, R.E., Castro, S.K. and Kramer, R.M. Acta Endocrinol. 1970. 63. 392/404. Enhancement of insulin release to acute glycemic stimulation with depression basal insulin production rates in insulinoma following diazoxide administration.
2. Blum, I., Doron, M., Laron, Z and Aston, A. Diabetes. 1975. 24. 535/537. Prevention of hypoglycemic attacks by propranolol in a patient suffering from insulinoma.

3. Broder, L.E. and Carter, S.K. Ann. Intern. Med. 1973. 79. 108/118. Pancreatic islet cell carcinoma. 2. Results of therapy with streptozotocin in 52 patients.
4. Curry, D.L., Benett, L.L. and Grodsky, G.M. Am. J. Physiol. 1968. 214. 174/178. Requirement for calcium ion in insulin secretion by the perfused rat pancreas.
5. Devis, G., Somers, G. and Malaisse, W.J. Diabetologia. 1977. 13. 531/536. Dynamics of calcium-induced insulin release.
6. Grodsky, G.M. and Benett, L.L. Diabetes. 1966. 15. 90/96. Cation requirement for insulin secretion in the isolated perfused pancreas.
7. Kohlhardt, M., Bauer, B., Krause, H. and Fleckenstein, A. Pflugers Arch. Physiol. 1972. 335. 309/322. Differentiation of the transmembrane Na and Ca channel in mammalian cardiac fibers by the use of specific inhibitors.
8. Lacy, P.E. and Kostianovsky, M. Diabetes. 1967. 16. 35/39. Method for the isolation of intact islets of Langerhans from the rat pancreas.
9. Malaisse-Lagae, F. and Malaisse, W.J. Endocrinology. 1971. 88. 72/80. Stimulus-secretion coupling of glucose-induced insulin release. 3. Uptake of ^{45}calcium by isolated islets of Langerhans.
10. Morgan, C.R. and Lazarow, A. Diabetes. 1963. 12. 115/126. Immunoassay of insulin: Two antibody systems. Plasma insulin levels of normal, subdiabetic and diabetic rats.
11. Naber, S.P., Mcdaniel, M.L. and Lacy, P.E. Endocrinology. 1977. 101. 686/693. The effect of glucose on the acute uptake and efflux of calcium-45 in isolated rat islets.
12. Sato, M., Nagao, T., Yamaguchi, I., Nakajima, H. and Kiyomoto, A. Arzneim Forsch. 1971. 21. 1338/1343. Pharmacological studies on a new 1, 5-benzothiazepine derivative (CRD-401).
13. Somers, G., Devis, G., Van Obberghen and Malaisse, W.J. Endocrinology. 1976. 114/124. Calcium antagonists and islet function. 2. Interaction of theophylline and verapamil.
14. Taniguchi, H., Murakami, K., Morita, S., Kazumi, T. Yoshino, G., Maeda, M. and Baba, S. Lancet 2. 1977. 501. Calcium antagonist (Diltiazem) for reversal of hypoglycaemic symptoms in insulinoma.

15. Yasuda, K., Hurukawa, Y., Okuyama, M., Kikuchi, M. and Yoshinaga, K. N. Engl. J. Med. 1975. 292. 501/504. Glucose tolerance and insulin secretion in patient with parathyroid disorders. Effect of serum calcium on insulin release.