EFFECT OF CALCIUM ANTAGONIST ON GLUCOSE-INDUCED INSULIN AND GLUCAGON SECRETION IN RATS WITH INSULIN-SECRETING TUMORS INDUCED BY STREPTOZOTOCIN AND NICOTINAMIDE

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INDEXING WORDS

calcium antagonist; insulin; glucagon; rat insulin-secreting tumor

SYNOPSIS

The effect of a calcium antagonist, diltiazem, on glucose-induced insulin and glucagon responses was examined in rats with insulin-secreting islet cell tumors induced by streptozotocin and nicotinamide.

The rats were orally given diltiazem 50 mg/kg body weight per day for one week. Diltiazem administration caused an inhibition of glucose-induced insulin release and an enhancement of both basal release and glucose-induced responses of glucagon though it caused no significant changes of blood glucose levels. The identical treatment, however, did not result in any deviation of blood glucose, plasma insulin and glucagon responses to glucose, except for increases of glucagon values at 2 hours following glucose load in normal control rats of comparable body weight.

These findings suggest that insulin-secreting tumors are supersensitive to deprivation of calcium.

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INTRODUCTION

We have shown that pancreatic islet cell tumors can be induced not only by streptozotocin with nicotinamide, but also by streptozotocin alone and streptozotocin with picolinamide,[14,15] and that induced neoplasms are capable of secreting insulin following oral glucose load.[15,28]

The tumor-bearing rats often showed hypoglycemia with hyperinsulinemia. In human insulinomas, hypoglycemic attacks can be prevented by diazoxide and streptozotocin, but may be followed by severe hypoglycemic crises.[2,3]

This study was undertaken in order to investigate the effect of the calcium antagonist, diltiazem,[22] on glucose-induced insulin and glucagon secretion in rats with insulin-secreting tumors induced by streptozotocin and nicotinamide.

MATERIALS AND METHODS

Induction of Insulin-Secreting Tumors.

Young male Wistar rats weighing 180 to 200 g were fasted overnight and injected intravenously with 65 mg/kg body weight of streptozotocin (Upjohn, Kalamazoo, Michigan, Lot 1613 E) preceded by 15 minutes by a single intraperitoneal injection of 500 mg/kg body weight of nicotinamide. Streptozotocin was dissolved in citrate buffer, pH 4.5, immediately before the injection and nicotinamide was dissolved in distilled water. Eleven rats, which had been treated with a combination of streptozotocin and nicotinamide one year ago, were used in this study. Pancreatic islet cell tumors were examined histologically by light microscope.

Administration of Diltiazem.

Diltiazem hydrochloride, d-3-acetoxycis-2, 3-dihydro-5-[2-(dimethylamino) ethyl]-2-(p-methoxyphenyl)-1, 5-benzo-thiazepin-4(5H)-one hydrochloride (Tanabe, Japan, Lot 670860), was dissolved in saline with a concentration of 5 mg/ml saline. Six tumor-bearing and 5 normal rats of comparable body weight were orally administered 25 mg/kg body weight of diltiazem twice a day, at 9 o'clock in the morning and at night, for one week under light ether anesthesia. Other five tumor-bearing rats were treated identically with the experimental rats except for the administra-

tion of diltiazem. They received oral administration of a similar volume of saline. The mean body weights of rats bearing tumors and of normal rats were 492±33 and 438±11 g, respectively.

Glucose Tolerance Test.

The animals were fasted overnight and 3 g/kg body weight of glucose in a 50% solution were administered into the stomach via a polyethylene tube under pentobarbital anesthesia. Blood samples (1 ml each) were obtained from the jugular vein before and 0.5, 1 and 2 hours, after glucose load. The blood was injected into tubes containing 0.1 ml of Trasylol (1,000 KIU/ml). The plasma was then separated by centrifugation and stored at -20°C until assayed. Blood glucose concentrations were measured by the method of Hoffman[11] using a Technicon Auto-Analyzer. Plasma insulin was measured by radioimmunoassay using the double-antibody system as rat insulin standard.[21] Plasma glucagon was measured by the dextran-charcoal radioimmunoassay,[1] using 30K antiserum.

The paired Students' "t" test was used for all statistical analyses.

RESULTS

As shown in the top portion of Fig. 1, the oral administration of diltiazem for one week resulted in significant decreases of plasma insulin values from 6.18±1.12 to 3.94±0.72 ng/ml at 0.5 hours after glucose load. Plasma insulin levels at 0, 1 and 2 hours, however, did not show any significant changes before and after diltiazem treatment. Furthermore, the treatment with the drug resulted in significant increases of the basal level as well as the glucose-induced response of glucagon (the top portion of Fig. 2). Before the calcium antagonist administration, the mean basal level of plasma glucagon was followed by a significant decrease at 0.5 hours and the suppression continued during oral glucose tolerance tests. On the contrary, plasma glucagon levels rose markedly (from 385±43 at 0 hour to 715±191 pg/ml at 0.5 hours) following glucose load after the drug administration. In contrast with changes to glucose-induced insulin and glucagon responses, the calcium antagonist had no influence on blood glucose levels during oral glucose tolerance tests (Fig. 3). The identical treatment, except for the administration of diltiazem in other

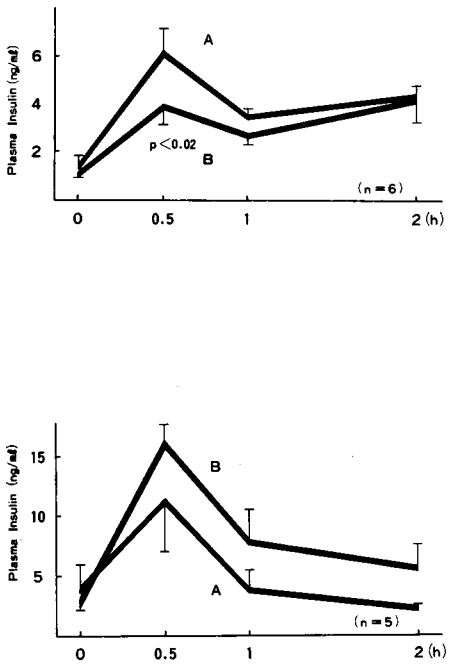

Fig. 1 Plasma insulin levels during oral glucose tolerance tests in rats with insulin-secreting tumors induced by streptozotocin and nicotinamide before (A) and after (B) the administration of the calcium antagonist (top) and saline (bottom). Six tumor-bearing rats were orally given diltiazem, 50 mg/kg body weight per day, for one week and other 5 tumor-bearing rats were treated identically except for the administration of the drug. Glucose-induced insulin release was significantly ($p<0.02$) supressed by the administration of the calcium antagonist. In this and subsequent figures vertical bars represent the standard errors of the means and the n in parentheses indicates the number of animals in each group.

tumor-bearing rats, had effects on neither blood glucose, plasma insulin nor glucagon values (the bottom portions of Figs. 1, 2 and 3). Insulin and glucagon responses of saline-treated tumor-bearing rats were similar before and after the administration of saline for one week, whereas these responses were different from

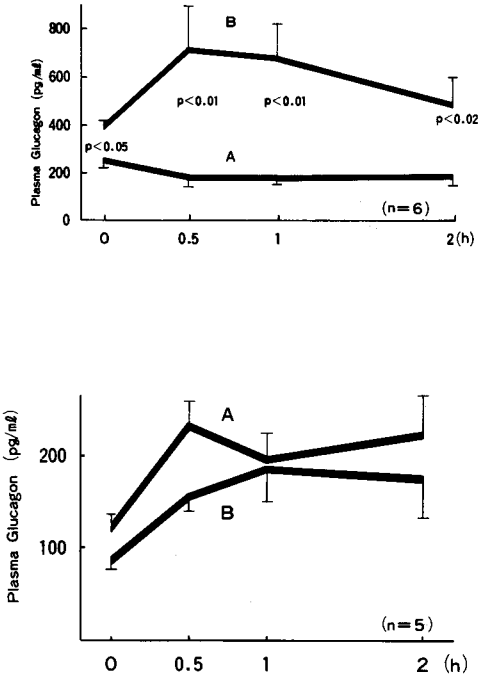

Fig. 2 Plasma glucagon levels during oral glucose tolerance tests before (A) and after (B) the calcium antagonist administration in tumor-bearing rats. The treatment with the calcium antagonist resulted in significant increases of the basal level as well as the glucose-induced responses of glucagon in tumor-bearing rats (top). On the contrary, tumor-bearing rats given saline instead of diltiazem showed no deviation of plasma glucagon levels (bottom).

those of the rats treated with diltiazem. In saline-treated rats, plasma insulin at 0.5 hours amounted to more than 10 ng/ml and basal glucagon levels were 119±17 and 88±12 pg/ml before and after treatment, respectively. Besides, plasma glucagon levels increased after glucose load in saline-treated rats.

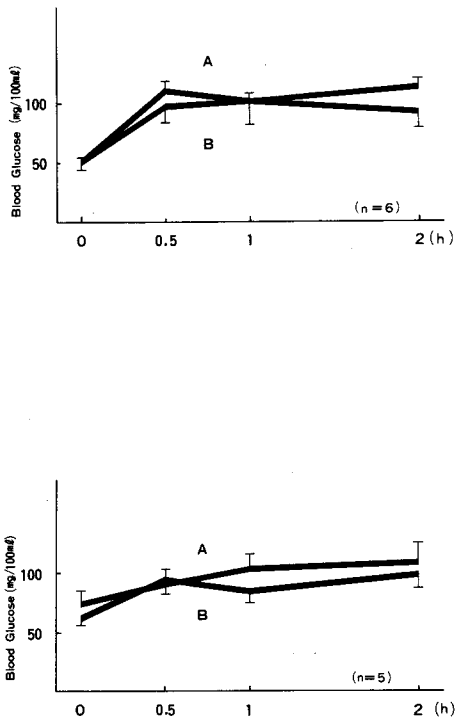

Fig. 3 The effect of diltiazem on blood glucose levels during oral glucose tolerance tests. The drug (top) as well as saline (bottom) had no effect on them. Abbreviations are the same as in Fig. 1.

An identical administration of diltiazem resulted in no significant deviation of blood glucose, plasma insulin and glucagon values during glucose tolerance tests in normal rats of comparable body weight, except for a significant increase of glucagon at 2 hours after treatment (Fig. 4).

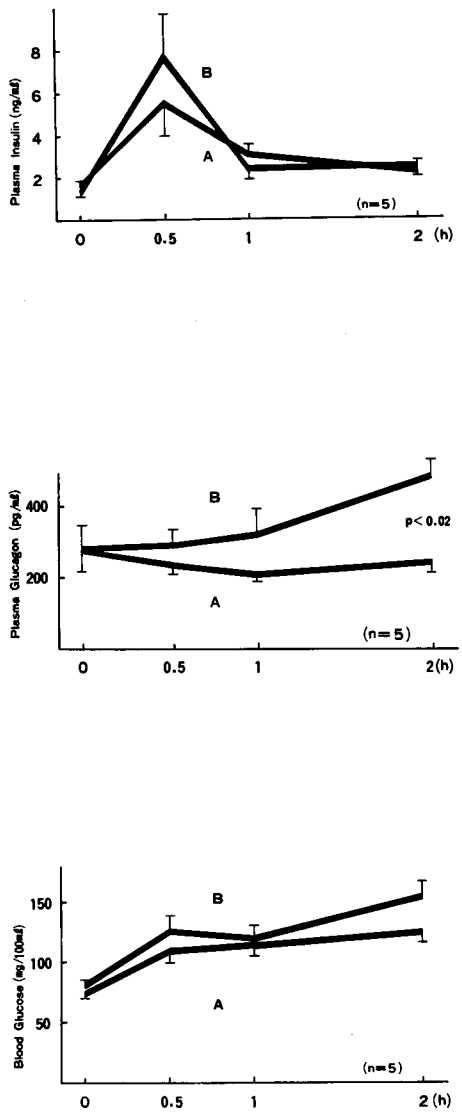

Fig. 4 The effect of the administration of the calcium antagonist on blood glucose, plasma insulin and glucagon levels during oral glucose tolerance tests in normal control rats of comparable body weight. The drug had no effect on these three parameters, except for an increase of the glucagon value at 2 hours after glucose load. Abbreviations are the same as in Fig. 1.

DISCUSSION

The data presented in Fig. 1 demonstrate that diltiazem, a calcium antagonist, provoked an inhibition of glucose-induced insulin responses in rats with insulin-secreting tumors induced by streptozotocin and nicotinamide. The calcium antagonist in the dosage used in this study, however, did not induce any significant change in insulin responses in normal rats of comparable body weight.

Calcium plays a critical role in the release of neurotransmitter substances and hormones.[24] This cation also influences insulin release, the secretory process being triggered by a cytosolic accumulation of the cation.[18,19] Calcium antagonists inhibiting transport of the cation into cells and/or mobilization of intracellular calcium suppress insulin release from normal islets,[5,20] while the calcium ionophore with the ability to transport the divalent cation across biological membranes stimulates it.[4] Thus, the data presented in Fig. 1 suggest that diltiazem suppressed insulin release from insulin-secreting tumors. It is not clear whether this difference in the effect of the agent on insulin responses seen in rats with insulin-secreting tumors as compared with control rats should be attributed to different mechanisms through which calcium played a role in insulin secretion. Gaeke et al.[9] reported that the infusion of calcium resulted in a simultaneous rise in plasma insulin and proinsulin and concurrent hypoglycemia in a patient with an islet cell tumor, and that after resection of the tumor an identical treatment caused no change in these parameters. The data shown in the present study and those reported by Gaeke et al. suggest that the tumor cells may have abnormal sensitivity to calcium ion with regard to insulin release.

The calcium antagonist enhanced both basal release and glucose-induced responses of glucagon in tumor-bearing rats. Plasma glucagon levels 2 hours after glucose load also increased in normal controls after treatment with diltiazem. The finding that there was no significant difference in glucagon responses before and after the saline treatment ruled out the possibility that these changes were a consequence of ether anesthesia. It is difficult to interpret the phenomenon mainly due to the following two reasons.

First, observations on glucagon secretion in rats with insulin-secreting tumors, as far as we know, are sparse and in human insulinoma, glucagon responses to glucose were found to show a variety of ensuing patterns.[8] In fact, tumor-bearing rats used in this study showed different glucagon responses. The mean basal glucagon level in rats given the calcium antagonist was twice higher than that in rats given saline alone and decreased significantly after oral glucose administration. By contrast, the glucose load resulted in a rise in glucagon levels in rats given saline alone. Secondly, although the key role of calcium in the release of insulin is well recognized, such a role of calcium in glucagon release remains a matter of debate. Calcium might alter the biophysical properties and stability of the membrane of A cells, or modify structural, electrical and functional coupling between A and B cells.[23] The cation might also interact with the glucose metabolism,[6,17] the cyclic-AMP system,[12,13] and the microtubular-microfilamentous system in the A cell.[7,16] There remains the possibility that the cation might alter the secretion of insulin and somatostatin from adjacent islet cells, these hormones possibly in turn influencing glucagon release.[27]

Recent studies suggest that glucagon stimulates insulin secretion[25] and that insulin inhibits glucagon secretion,[26] providing the existence of an intra-islet insulin-glucagon feedback system. We presume, from these observations, that glucagon secretion might be suppressed by a large amount of insulin released from the tumors and that the enhancement of glucagon secretion might result from the inhibition of insulin release from insulin-secreting tumors.

Finally, the present study indicates that insulin-secreting tumors may be super-sensitive to deprivation of calcium.

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