THE EARLY PHASE OF CALCIPENIA-INDUCED PARATHYROID HORMONE SECRETION IS BLUNTED IN VASCULARY PERFUSED PARATHYROID GLANDS OF STREPTOZOTOCIN-DIABETIC RATS

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SUMMARY - Objectives: To study effects of diabetes mellitus on parathyroid hormone (PTH) secretion, the rat parathyroid glands were perfused via the bilateral carotic arteries.

Material and Methods: Diabetic rats showed plasma glucose levels above 30 mmol/l, but no change in plasma PTH concentration, 14 days after an administration of 70 mg/kg of streptozotocin. Krebs-Ringer bicarbonate buffer containing 0.1 % bovine serum albumin was used for perfusate. Per fusate calcium was lowered from 2.5 mmol/l to 0.5 mmol/l.

Results: In the normal rat parathyroid glands, PTH secretion was promptly evoked by calcipenia (from below 0.25 ng for 2.5 min in the perfusion with normal calcium to 0.73 ± 0.17 ng during calcipenia time 0-2.5 min), and slightly decreased (0.51 ± 0.12 ng during calcipenia time 2.5-5 min), and then increased (0.75 ± 0.16 ng during calcipenia time 7.5-10 min). Diabetes abolished the early phase of PTH secretion (below 0.25 ng during calcipenia time 0-2.5 min), but did not affect the late phase of PTH secretion. Insulin treatment with 25 U/kg daily for 14 days completely normalized the early phase of PTH secretion.

Conclusion: It is suggested that an insulin-deficient diabetes, not quantitatively but qualitatively, impairs the PTH secretion.

Key-words: rat parathyroid gland perfusion, parathyroid hormone secretion, diabetes mellitus.
Diabetes mellitus causes a marked change in calcium and phosphorus metabolism in the bone and kidney [1-3]. Blood parathyroid hormone (PTH) levels in diabetics are reported to be lowered [4], to be elevated [5], or to be not affected [6]. In cultured parathyroid cells isolated from non-diabetic cattle, both a high concentration of glucose and a removal of insulin reduced PTH secretion [7]. However, there are few studies to directly observe the PTH secretion in the parathyroid gland of diabetic animals. The in vivo study suggested that hypocalcemia-induced PTH secretion was composed of the early pulsatile secretion and the late tonic secretion [8]. In the present study, the parathyroid glands together with the thyroid glands were isolated and vascularly perfused to examine the time course of calcipenia-stimulated PTH secretion in the streptozotocin (STZ)-induced diabetic rats.

**Materials and Methods**

**Animals** – Male Wistar rats, weighing 200-220 g, were divided into three groups; normal control, STZ-diabetic, and insulin-treated diabetic rats. Fourteen days after an intraperitoneal administration with 70 mg/kg of STZ, rats showing plasma glucose levels above 30 mmol/l were used as the STZ-diabetic rats. Insulin-treated STZ-diabetic rats were treated with a daily injection of 25 U/kg lente-type human insulin 14 days into both cannulae. The bilateral common carotic arteries were ligated at a more rostral level than the thyroid glands and parathyroid glands associated with the cervical spine and muscles, and the carotic arteries and veins were isolated en bloc. The carotic veins were cut open, and venous effluent was collected every 2.5 min. The perfusion system was maintained at 37 °C.

**Perfusate** – Perfusate was Krebs-Ringer bicarbonate buffer (pH 7.4) consisted of 2 % dextran T70, 0.1 % bovine serum albumin, 5 mmol/l glucose, 2.5 mmol/l KCl, 1.2 mmol/l KH2PO4, 1.2 mmol/l MgSO4, 25 mmol/l NaHCO3, and 118 mmol/l NaCl-2.5 mmol/l CaCl2, or 122 mmol/l NaCl-0.5 mmol/l CaCl2. Perfusate was equilibrated with 95 % O2-5 % CO2 gas.

**Perfusion procedures** – Rats, were fed ad libitum, were anesthetized with an intraperitoneal pentobarbital sodium. The neck and chest were opened. Each of right and left common carotic arteries were cannulated through a slit made on the aorta. Perfusate was pumped at 0.5 ml/min into each cannula, i.e., at 1 ml/min into both cannulae. The bilateral common carotic arteries were ligated at a more rostral level than the thyroid glands. The bilateral thyroid glands and parathyroid glands associated with the cervical spine and muscles, and the carotic arteries and veins were isolated en bloc. The carotic veins were cut open, and venous effluent was collected every 2.5 min. The perfusion system was maintained at 37 °C.

**Analyses** – Serum PTH was determined by a radioimmunoassay kit (Yamasa, Tokyo, Japan) consisting of the human PTH standard, 125I-human PTH (43-68), and an antibody against bovine PTH (1-84), which was known to cross react with rat PTH [9]. A radioimmunoassay kit for human insulin was purchased from Shionogi (Osaka, Japan). Insulin in the human insulin-treated diabetic rat was measured by use of the human insulin standard. Insulin in the control and diabetic rats was determined by use of the rat insulin standard (Cosmo-Bio, Tokyo). Student’s t-test was used for statistics.

**Results and Discussion**

In the present study, plasma calcium, phosphorus, and PTH levels were not different among the control, diabetic and insulin-treated diabetic rats (Table I). As shown in Fig. 1, calcipenia-induced PTH secretion was biphasic in the perfused parathyroid glands. In the normal rat, PTH was promptly secreted during calcipenia from below 0.25 ng for 2.5 min with normal calcium to 0.73 ± 0.17 ng for 2.5 min, and slightly decreased during calcipenic perfusion time 2.5-5 min (0.51 ± 0.12 ng for 2.5 min), and then increased again (0.75 ± 0.16 ng for 2.5 min during calcipenia time 7.5-10 min). Diabetes abolished the early phase of PTH secretion (below 0.25 ng for 2.5 min during calcipenia time 0-2.5 min), but did not affect the late phase of PTH secretion (0.64 ± 0.14 ng for 2.5 min during calcipenia time 7.5-10 min). Insulin treatments partly normalized the blood glucose level, and completely normalized the early phase of PTH secretion. It is suggested that calcipenia-induced PTH secretion is qualitatively impaired by a severe insulinopenia in diabetes. Sugimoto et al. [7] showed that PTH secretion was suppressed in bovine parathyroid cells cultured without insulin. However, it is possible that the insulin effect on cultured cells may be an effect as growth factor.

PTH has the key role to maintain the blood calcium concentration within a very narrow range. It is emphasized that the “set point” of PTH secretion, i.e., the calcium concentration half-maximally inhibiting PTH secretion, is important to keep the normal calcium level [10]. Many studies of the relationship between calcium and PTH levels suggest that the set point of PTH release is not impaired in diabetic states. Serum calcium was lowered and PTH was elevated in diabetic rats 3 weeks after STZ treatments [5]. Rats 7 weeks after STZ administration had an elevated serum calcium and a lowered PTH level [4]. Both serum calcium and PTH levels were normal in the diabetic patients [6]. In the present study, serum calcium and PTH levels were not changed by the insulinopenic diabetes of 2 weeks duration.

The present vascular perfusion study demonstrates that the calcipenia-induced PTH secretion consists of...
the quick response of PTH secretion within 2.5 min and the slow response 5 min after a lowering of calcium. The early and pulsatile PTH secretion and the late and tonic PTH secretion were observed in normal subjects infused with sodium citrate [8]. The mechanism by which insulinopenic diabetes blunts the early phase of PTH secretion is unknown. PTH secretion was augmented by beta-agonist, and was decreased by alpha-agonist [11]. PTH response to an EDTA infusion was decreased in the rat with superior cervical ganglionectomy [12]. It is possible that the parathyroid function is influenced by the injury of intraglandular sympathetic nervous system by diabetes. With this respect, it is necessary to investigate whether a normal response could be restored by beta-mimetics, or to make histological analysis of the glands in further studies.

REFERENCES


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TABLE I. Metabolic conditions in normal control, STZ-diabetic, and human insulin-treated diabetic rats.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Diabetic</th>
<th>Insulin-treated diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/l)</td>
<td>7.1 ± 1.0</td>
<td>34.6 ± 3.5*</td>
<td>15.2 ± 3.8*</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>4.3 ± 0.9a</td>
<td>0.2 ± 0.1b&lt;1</td>
<td>4.0 ± 1.1b</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.3 ± 0.2</td>
<td>3.3 ± 0.2</td>
<td>3.2 ± 0.2</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>10.2 ± 0.4</td>
<td>10.0 ± 0.6</td>
<td>10.0 ± 0.6</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>9.0 ± 0.5</td>
<td>9.1 ± 0.6</td>
<td>9.0 ± 0.5</td>
</tr>
<tr>
<td>PTH (pg/ml)</td>
<td>330 ± 81</td>
<td>298 ± 72</td>
<td>285 ± 70</td>
</tr>
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Insulin immunoreactivity is determined by use of rat insulin standard \( ^* \) and the human insulin standard \( ^b \). Data are mean ± SD \( (n = 5) \). \(^* \) \( P < 0.01 \) vs control rats.

FIG 1. Rat parathyroid glands were perfused with Krebs-Ringer bicarbonate buffer added with 0.1% albumin. Perfusate calcium was lowered from 2.5 to 0.5 mmol/l, and then was recovered to 2.5 mmol/l. PTH was biphasically secreted in the control (□) and the insulin-treated diabetic rat (●). The early phase of PTH secretion was defective in the diabetic rat (○). Data are mean ± SD \( (n = 5) \). \(^* \) \( P < 0.05 \) vs control rat experiments.