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Tetrahydrobiopterin Regulates L-tyrosine-induced Elevation of Cytosolic Calcium Concentration in Insulin-secreting Cells

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Abstract

We examined the role of endogenous tetrahydrobiopterin (BH₄) on L-tyrosine-induced increase in cytosolic Ca²⁺ concentration ([Ca²⁺]_i) in the β-cell line, HIT-T15. Addition of L-tyrosine to the cells increased [Ca²⁺]_i in a concentration-dependent manner. Moreover, the L-tyrosine-induced [Ca²⁺]_i elevation was blocked by L-3,4-dihydroxyphenylalanine (L-DOPA). Under low levels of BH₄ by treatment with 2,4-diamino-6-hydroxypyrimidine (DAHP), an inhibitor of BH₄ biosynthesis, elevation of [Ca²⁺]_i by L-tyrosine was stimulated. Moreover, co-pretreatment with sepiapterin, a precursor of BH₄ biosynthesis, reduced the decrease of BH₄ content by DAHP, and suppressed L-tyrosine-induced [Ca²⁺]_i elevation in DAHP-pretreated cells. These findings suggest that BH₄ negatively regulates L-tyrosine-induced [Ca²⁺]_i elevation via the production of L-DOPA in β-cells.

Key words: tetrahydrobiopterin, calcium, L-tyrosine, β-cell

Introduction

Numerous insulinotropic substances including glucose, fatty acid and amino acid have been shown to increase insulin secretion with elevation of [Ca²⁺]_i through the plasma membrane Ca²⁺ channels including L-type voltage-operated Ca²⁺ channel and transient receptor potential TRPM2 channel (1-5). Recent studies showed that insulin secretion is controlled by Ca²⁺ release from intracellular stores in the endoplasmic reticulum as well as Ca²⁺ influx from extracellular spaces (6,7). Thus, it is widely accepted that the increase in [Ca²⁺]_i is essential for the beginning of insulin secretion in pancreatic β-cells.

Tetrahydrobiopterin (BH₄) is an essential cofactor not only for nitric oxide (NO) synthases but also for aromatic L-amino acid hydroxylases including tyrosine hydroxylase (8,9). In diabetic conditions, the content of BH₄ is decreased in vessels (10) and brain (11). It is possible that BH₄ content may be decreased in diabetic β-cells. In our previous study, the decrease in BH₄ content may have promoted the L-arginine-induced [Ca²⁺]_i elevation through the reduction of NO production (12). L-Tyrosine, a substrate of tyrosine hydroxylase, is known to stimulate insulin secretion in

the presence of glucose (13). Moreover, it is known that L-DOPA, a product of tyrosine hydroxylase, reduces glucose-induced insulin secretion (13). Therefore, it is possible that L-DOPA may also reduce L-tyrosine-induced insulin secretion. We hypothesize that the decrease in BH₄ content also stimulates L-tyrosine-induced insulin secretion through the reduction of tyrosine hydroxylase activities. In the present study, we examined the role of BH₄ on L-tyrosine-induced [Ca²⁺]_i elevation using HIT-T15 cells, from a hamster pancreatic β-cell line.

Materials and Methods

Cell culture

Hamster pancreatic β-cell line, HIT-T15 cells (Dainippon Pharmaceutical Co., Osaka, Japan; passages 70-85) were cultured in Ham's F12 medium containing 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 µg/mL streptomycin.

Measurement of changes in [Ca²⁺]_i

Cells on coverslips were loaded with fura-2 in HEPES-buffered saline (HBS) containing (in mM) 107

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NaCl, 6 KCl, 1.2 MgSO₄, 2 CaCl₂, 11.5 glucose, 20 HEPES, adjusted to pH 7.4 with NaOH. Cells were incubated with HBS containing 5 μ M fura-2/AM (Dojindo Laboratories, Kumamoto, Japan) and 0.1% bovine serum albumin at 37°C for 40 min. The fura-2-loaded cells were then incubated with HBS for 20 min. The coverslips were then placed in a perfusion chamber mounted on the stage of the microscope. Fluorescence images of the cells were recorded and analyzed with a video images analysis system (MetaFluor, Nippon Roper, Osaka, Japan). Fluorescence emission at 510 nm was monitored with excitation at 340 nm and 380 nm. The pairs of 340 nm and 380 nm fluorescence images were captured every 5 sec and the 340/380 nm ratio was calculated after subtraction of background fluorescence. Data from all measurements are expressed as the fluorescence ratio of 340/380 nm.

Measurement of total biopterin content

HIT-T15 cells in 6-well plates (3.5 $\times$ 10⁵ cells/well) were directly treated with 0.02 M KI/I₂ in 0.1 M HCl, and total biopterin (BH₄ plus 7,8-dihydrobiopterin (BH₂) plus biopterin) was measured, as previously described (14).

Materials

L-Tyrosine and L-DOPA were from Wako Pure Chemicals (Osaka, Japan). 2,4-Diamino-6-hydroxypyrimidine (DAHP) was from Aldrich Chemical Co. (Tokyo, Japan). Sepiapterin was from Funakoshi (Tokyo, Japan). All other reagents were of the highest grade commercially available.

Statistical analysis

Data are presented as means $\pm$ S.E.M. of *n* observations. The statistical significance of observed differences was determined by analysis of variance followed by Bonferroni's method. Differences between means were considered significant when *P* was less than 0.05.

Results

Changes in [Ca²⁺]_i by L-tyrosine

We examined whether L-tyrosine (5 mM), the substrate of tyrosine hydroxylase, induces Ca²⁺ response in HIT-T15 cells. Addition of L-tyrosine (0.1–10 mM) to HIT-T15 cells increased [Ca²⁺]_i in a concentration-dependent manner (Figure 1). Moreover, the L-tyrosine-induced [Ca²⁺]_i elevation was inhibited by L-DOPA, the product of tyrosine hydroxylase (Figure 2).

Effect of BH₄ on L-tyrosine-induced Ca²⁺ response

To determine whether L-tyrosine-induced Ca²⁺

Figure 1 L-Tyrosine-induced [Ca²⁺]_i elevation in HIT-T15 cells. Cytosolic Ca²⁺ was measured using fura-2 as a Ca²⁺ indicator. In A, cells were treated with L-tyrosine (5 mM). [Ca²⁺]_i is expressed as a fura-2 fluorescence ratio (340/380 nm). In B, the effect of L-tyrosine (0.1–10 mM) in [Ca²⁺]_i was measured in individual cells. The ratio in panel B indicates the absolute changes of ratio intensity (Δ ratio (340/380 nm) = peak ratio - resting ratio). Results are the means $\pm$ SEM of 15–43 cells.

response is regulated by intracellular BH₄ content, the effects of DAHP, the inhibitor of BH₄ synthesis, and sepiapterin, the precursor of BH₄ biosynthesis, were examined. As shown in Figure 3A, we measured the total biopterin content (BH₄ plus more oxidized forms) in HIT-T15 cells, which were treated with DAHP (1 mM) and/or sepiapterin (1–100 μ M) for 6 h. Treatment with DAHP reduced intracellular biopterin content (Figure 3A), and its decrease of intracellular biopterin content was recovered to control levels with co-treatment with sepiapterin (10 μ M). L-Tyrosine-induced [Ca²⁺]_i elevation was stimulated in DAHP-treated cells, and its stimulation response was reduced by co-treatment with sepiapterin (10 μ M) (Figure 3B).

Discussion

It is well known that [Ca²⁺]_i elevation stimulates insulin secretion in pancreatic β -cells (1–7). In the present study, we measured real-time changes in [Ca²⁺]_i in a hamster pancreatic b-cell line, HIT-T15 cells, in

Figure 2 Effect of L-DOPA on L-tyrosine-induced [Ca²⁺]_i elevation in HIT-T15 cells. In A, cells were treated with L-tyrosine (5 mM) in the presence of L-DOPA (5 mM). [Ca²⁺]_i is expressed as a fura-2 fluorescence ratio (340/380 nm). In B, the effect of L-DOPA on the peak levels of L-tyrosine (5 mM)-induced [Ca²⁺]_i elevation was measured. Cells were pretreated with L-DOPA (5 mM) for 5 min. The ratio in panel B indicates the absolute changes of ratio intensity (Δ ratio (340/380 nm) = peak ratio - resting ratio). Results are the means $\pm$ SEM of 30-55 cells. *Significant changes in comparison to control group ($p < 0.05$). #Significant changes in comparison to L-tyrosine (5 mM)-treated group ($p < 0.05$).

order to estimate the role of BH₄, which is the cofactor of tyrosine hydroxylase, on insulin secretion by L-tyrosine. The decrease in BH₄ content promoted L-tyrosine-induced [Ca²⁺]_i elevation. Its response was significantly reduced to control levels by co-treatment with sepiapterin. These findings suggest that endogenous BH₄ may negatively regulate L-tyrosine-induced [Ca²⁺]_i elevation.

Tyrosine hydroxylase is present in many kinds of animal tissues (9). Lindstrom and Sehlin (13) reported that L-DOPA decreases glucose-induced insulin secretion from the pancreatic islet. L-Tyrosine is known to stimulate glucose-induced insulin secretion (13). In this study, we observed that treatment with L-DOPA reduces the L-tyrosine-induced [Ca²⁺]_i elevation in the presence of glucose. It is known that L-DOPA is rapidly converted to dopamine by aromatic L-amino acid

Figure 3 Effects of intracellular biopterin on L-tyrosine-induced [Ca²⁺]_i elevation in HIT-T15 cells. In A, cells were incubated with DAHP (1 mM) and sepiapterin (1-100 μ M) for 6 h. Total biopterin levels were determined. In B, the effects of pretreatment with DAHP (1 mM) and/or DAHP (1 mM) and sepiapterin (10 μ M) on the peak levels of L-tyrosine (5 mM)-induced [Ca²⁺]_i elevation was measured. Cells were pretreated with DAHP (1 mM) and/or sepiapterin (10 μ M) for 6 h. The ratio in panel B indicates the absolute changes of ratio intensity (Δ ratio (340/380 nm) = peak ratio - resting ratio). Results are the average of 53-74 cells. Results are the means $\pm$ SEM of 30-55 cells. *Significant changes in comparison to control group ($p < 0.05$). #Significant changes in comparison to L-tyrosine (5 mM)-treated group ($p < 0.05$). \$Significant changes in comparison to DAHP (1 mM)-treated group ($p < 0.05$).

decarboxylase (14,15). L-Tyrosine hydroxylase activity is regulated by the feedback inhibition of dopamine (9). Under the decrease in BH₄ content, the conversion of L-tyrosine to L-DOPA may be reduced. Thus, the production of dopamine from L-DOPA is also likely to be decreased. Therefore, BH₄ may negatively control L-tyrosine-induced [Ca²⁺]_i elevation through production of dopamine in the presence of glucose. The inhibition mechanism of L-DOPA on L-tyrosine-induced [Ca²⁺]_i elevation is needed to examine in the future.

Shinozaki *et al.* (10) reported that BH₄ content in vessels is decreased in fructose-fed rats (a model of insulin resistance with endogenous hyperinsulinemia).

The activities of GTP cyclohydrolase I (GTPCH), the enzyme of BH₄ synthesis, and dihydropteridine reductase (DHPR), the recycling enzyme of BH₄, have been shown to be decreased in diabetes (17). In streptozotocin-induced diabetic brain, DHPR activity is also reduced (11). Under diabetes mellitus, BH₄ content may be decreased by downregulating the key enzymes of BH₄ synthesis. In diabetic β -cells, BH₄ content also seems to be decreased. We previously reported that insulin stimulates BH₄ synthesis in endothelial cells (18,19). In this study, the decrease in BH₄ content stimulated L-tyrosine-induced [Ca²⁺]_i elevation. We previously also suggested that a decrease in BH₄ content promotes the L-arginine-induced [Ca²⁺]_i elevation (12). Therefore, we speculated that the decrease in BH₄ content under diabetic conditions, may stimulate BH₄ synthesis via the enhancement of insulin secretion as one of the cell defense system, since BH₄ has antioxidative activities (20,21).

In conclusion, our findings suggest that endogenous BH₄ may negatively regulate L-tyrosine-induced insulin secretion in β -cells through the reduction of L-tyrosine-induced [Ca²⁺]_i elevation. The physiological role of BH₄ in β -cells should be examined in the future.

References

- Nelson, TY, Gaines, KL, Rajan, AS, Berg, M, Boyd, AE, 3rd. Increased cytosolic calcium. A signal for sulfonylurea-stimulated insulin release from beta cells. *J Biol Chem* 1987; 262: 2608-2612.
- Komatsu, M, Yokokawa, N, Takeda, T, Nagasawa, Y, Aizawa, T, Yamada, T. Pharmacological characterization of the voltage-dependent calcium channel of pancreatic B-cell. *Endocrinology* 1989; 125: 2008-2014.
- Regazzi, R, Li, GD, Deshusses, J, Wollheim, CB. Stimulus-response coupling in insulin-secreting HIT cells. Effects of secretagogues on cytosolic Ca²⁺, diacylglycerol, and protein kinase C activity. *J Biol Chem* 1990; 265: 15003-15009.
- Itoh, Y, Kawamata, Y, Harada, M, Kobayashi, M, Fujii, R, Fukusumi, S, Ogi, K, Hosoya, M, Tanaka, Y, Uejima, H, Tanaka, H, Maruyama, M, Satoh, R, Okubo, S, Kizawa, H, Komatsu, H, Matsumura, F, Noguchi, Y, Shinohara, T, Hinuma, S, Fujisawa, Y, Fujino, M. Free fatty acids regulate insulin secretion from pancreatic β -cells through GPR40. *Nature* 2003; 422: 173-176.
- Togashi, K, Hara, Y, Tominaga, T, Higashi, T, Konishi, Y, Mori, Y, Tominaga, M. TRPM2 activation by cyclic ADP-ribose at body temperature is involved in insulin secretion. *EMBO J* 2006; 25: 1804-1815.
- Takasawa, S, Akiyama, T, Nata, K, Kuroki, M, Tohgo, A, Noguchi, N, Kobayashi, S, Kato, I, Katada, T, Okamoto, H. Cyclic ADP-ribose and inositol 1,4,5-trisphosphate as alternate second messengers for intracellular Ca²⁺ mobilization in normal and diabetic β -cells. *J Biol Chem* 1998; 273: 2497-2500.
- Kato, I, Yamamoto, Y, Fujimura, M, Noguchi, N, Takasawa, S, Okamoto, H. CD38 disruption impairs glucose-induced increases in cyclic ADP-ribose, [Ca²⁺]_i, and insulin secretion. *J Biol Chem* 1999; 274: 1869-1872.
- Shimizu, S, Ishii, M, Momose, K, Yamamoto, T. Role of tetrahydrobiopterin in the function of nitric oxide synthase, and its cytoprotective effect. *Int J Mol Med* 1998; 2: 533-540.
- Nagatsu, T. Tyrosine hydroxylase: human isoforms, structure and regulation in physiology and pathology. *Essays Biochem* 1995; 30: 15-35.
- Shinozaki, K, Kashiwagi, A, Nishio, Y, Okamura, T, Yoshida, Y, Masada, M, Toda, N, Kikkawa, R. Abnormal biopterin metabolism is a major cause of impaired endothelium-dependent relaxation through nitric oxide/O²- imbalance in insulin-resistant rat aorta. *Diabetes* 1999; 48: 2437-2445.
- Hamon, CG, Cutler, P, Blair, JA. Tetrahydrobiopterin metabolism in the streptozotocin induced diabetic state in rats. *Clin Chim Acta* 1989; 181: 249-253.
- Watabe, T, Ishii, M, Mori, Y, Shimizu, S, Kiuchi, Y. Decrease in tetrahydrobiopterin content promotes L-arginine-induced elevation of cytosolic calcium concentration in insulin-secreting cells. *Pteridines* 2003; 14: 119-125.
- Lindstrom, P, Schlin, J. Mechanisms underlying the effects of 5-hydroxytryptamine and 5-hydroxytryptophan in pancreatic islet. A proposed role for L-aromatic amino acid decarboxylase. *Endocrinology* 1983; 112: 1524-1529.
- Shimizu, S, Shiota, K, Yamamoto, S, Miyasaka, Y, Ishii, M, Watabe, T, Nishida, M, Mori, Y, Yamamoto, T, Kiuchi, Y. Hydrogen peroxide stimulates tetrahydrobiopterin synthesis through the induction of GTP-cyclohydrolase I and increases nitric oxide synthase activity in vascular endothelial cells. *Free Radic Biol Med* 2003; 34: 1343-1352.
- Ericson, LE, Hakanson, R, Lundquist, I. Accumulation of dopamine in mouse pancreatic B cell following injection of L-DOPA. Localization to secretory granules and inhibition of insulin secretion. *Diabetologia*. 1977;13:117-124.
- Ahren, B, Lundquist, I. Effects of L-dopa-induced

- dopamine accumulation on ⁴⁵Ca²⁺ efflux and insulin secretion in isolated rat islets. *Pharmacology* 1985; 30: 71-82.
- 17 Shinozaki, K., Hirayama, A., Nishio, Y., Yoshida, Y., Ohtani, T., Okamura, T., Masada, M., Kikkawa, R., Kodama, K., Kashiwagi, A. Coronary endothelial dysfunction in the insulin-resistant state is linked to abnormal pteridine metabolism and vascular oxidative stress. *J Am Coll Cardiol* 2001; 38: 1821-1828.
- 18 Ishii, M., Shimizu, S., Nagai, T., Kiuchi, Y., Yamamoto, T. Insulin stimulates tetrahydrobiopterin synthesis in mouse brain microvascular endothelial cells. *Pteridines* 1999; 10: 213-216.
- 19 Ishii, M., Shimizu, S., Nagai, T., Shiota, K., Kiuchi, Y., Yamamoto, T. Stimulation of tetrahydrobiopterin synthesis induced by insulin: possible involvement of phosphatidylinositol 3-kinase. *Int J Biochem Cell Biol* 2001; 33: 65-73.
- 20 Kojima, S., Ona, S., Iizuka, I., Arai, T., Mori, H., Kubota, K. Antioxidative activity of 5,6,7,8-tetrahydrobiopterin and its inhibitory effect on paraquat-induced cell toxicity in cultured rat hepatocytes. *Free Radic Res* 1995; 23: 419-430.
- 21 Ishii, M., Shimizu, S., Momose, K., Yamamoto, T. Reduction by tetrahydrobiopterin of H₂O₂-induced endothelial cell injury. *Pharmacol Toxicol* 1998; 82: 280-286.