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Effect of Oxytocin on Cytosolic Calcium Concentration of Alpha and Beta Cells in Pancreas

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***Abstract*—**Oxytocin is a nine-amino acid peptide synthesized in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus. Oxytocin promotes contraction of the uterus during birth and milk ejection during breast feeding. Although oxytocin receptors are found predominantly in the breasts and uterus of females, many tissues and organs express oxytocin receptors, including the pituitary, heart, kidney, thymus, vascular endothelium, adipocytes, osteoblasts, adrenal gland, pancreatic islets, and many cell lines. On the other hand, in pancreatic islets, oxytocin receptors are expressed in both α-cells and β-cells with stronger expression in α- cells. However, to our knowledge there are no reports yet about the effect of oxytocin on cytosolic calcium reaction on α and β-cell. This study aims to investigate the effect of oxytocin on α-cells and β-cells and its oscillation pattern. Islet of Langerhans from wild type mice were isolated by collagenase digestion. Isolated and dissociated single cells either α-cells or β-cells on coverslips were mounted in an open chamber and superfused in HKRB. Cytosolic concentration ([Ca2+]i) in single cells were measured by fura-2 microfluorimetry. After measurement of [Ca2+]i, α-cells were identified by subsequent immunocytochemical staining using an anti-glucagon antiserum. In β-cells, the [Ca2+]i increase in response to oxytocin was observed only under 8.3 mM glucose condition, whereas in α-cells, [Ca2+]i an increase induced by oxytocin was observed in both 2.8 mM and 8.3 mM glucose. The oscillation incidence was induced more frequently in β-cells compared to α-cells. In conclusion, the present study demonstrated that oxytocin directly interacts with both α-cells and β-cells and induces increase of [Ca2+]i and its specific patterns.

***Keywords*—**α-cells, β-cells, cytosolic calcium concentration, oscillation, oxytocin.

I. Introduction

OXYTOCIN (Oxt) is released peripherally after being shuttled to the pituitary [1], and promotes mammalian labor and lactation [2]. Furthermore, Oxt is also plays important role to regulate feeding behavior and body weight [3]-[6], as well as social behavior [7] including trust [8] and mother-infant bonding [9]. A previous study placed Oxt in the anorexigenic circuit from the PVN to the NTS of brain stem in rats [10].

We have previously shown that subchronic peripheral Oxt treatment through a subcutaneously (sc) implanted osmotic minipump reduces hyperphagia and obesity in high fat diet (HFD)-fed obese mice [6], and that the Oxt administered via IP and nasal routes similarly reduce hyperphagia [11]. Zhang et al. recently reported that nasal administration of Oxt decreases body weight in obese subjects [12]. Ott et al. showed that nasal Oxt treatment reduces reward-driven food intake [13]. Nasal administration of Oxt was also recently shown to improve social behavior in autism [14].

It has been reported that the release of islet hormones is regulated by the paracrine effects [15]; insulin inhibits glucagon release and glucagon potentiates insulin release [16], [17]. However, direct action of Oxt on islet α-cells and β-cells remain to be elucidated. The present study aimed to clarify whether Oxt directly interacts with and active α-cells and β-cells. This was achieved by studying direct action of Oxt on cytosolic free Ca^{2+} concentration ($[Ca^{2+}]i$) in single islet cells.

II. Material and Methods

A. Animal

Male wild-type C57BL/6J mice 10-12 weeks old were housed in accordance with our institutional guidelines and with the Japanese Physiological Society's guidelines for animal care.

B. Preparation of Pancreatic Islets and Single Islet Cells

Mice were anesthetized by pentobarbital (ip) at dosage 80 mg/kg. Collagenase at 1.05 mg/ml (Sigma-Aldrich) was dissolved in 5 mmol/l Ca^{2+} containing HEPES-added Krebs-Ringer bicarbonate buffer (HKRB) solution and injected into the common bile duct. The HKRB solution consist of 129 mmol NaCl, 5 mmol/l $NaHCO_3$, 4.7 mmol/l KCl, 1.2 mmol/l KH_2PO_4, 2 mmol/l $CaCl_2$, 1.2 mmol/l $MgSO_4$, and 10 mmol/l HEPES, at pH 7.4 with NaOH. HKRB was added with 0.1% BSA. The pancreas was dissected out and incubated at 37°C for 16 minutes. Islets were collected and dispersed into single cells in Ca^{2+} -free HKRB and maintained in short-term culture up to 2 days (37°C). It is important to keep the atmosphere of 5% CO_2 and 95% air in Eagle's minimal essential medium (5.6 mmol/l glucose supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin [18]

C. Measurements of $[Ca^{2+}]_i$ in Mice Single α-Cells and β-Cells

Single cells of α-cells or β-cells on coverslips were superfused in HKRB. $[Ca^{2+}]_i$ in single cells were measured by fura-2 microfluorimetry with wavelength at 340/380 nm and emission at 510 nm using a cooled charge-coupled device

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camera. The ratio image was produced on an AquaCosmos system (Hamamatsu Photonics, Hamamatsu, Japan).

D.Immunocytochemistry and Identification of Single Islet α-Cells

Single islet of α-cells were identified by immunocytochemical staining using an anti-glucagon antiserum after measurement of $[Ca^{2+}]_i$. The cells on the coverslip were fixed with 4% paraformaldehyde overnight. They were washed with PBS and then treated with H_20_2 3% for 10 min. After 10 min, cells were washed with PBS and treated with 10% normal goat serum and 1% BSA in PBS for 30 min. Glucagon antibodies (final dilution, 1:2000) were used as primary antibody. Cells were treated with glucagon antibody for 6 hours, followed by incubation with biotinylated anti-mouse IgG (Vector laboratories, Inc., Burlingame, CA) for 40 min and then with avidin-biotin complex for 40 min. After application of Diaminobenzidine (DAB), the reaction was stopped with PBS.

Correlation of the $[Ca^{2+}]_i$ and immunocytochemical data was carried out as previously reported [19] ; the phase contrast photographs of islet cells on coverslips in the microscope field taken at the end of $[Ca^{2+}]_i$ measurement were compared with the photographs of islet cells on coverslips after immunocytochemical staining. $[Ca^{2+}]_i$ data were obtained only from the immunocytochemically identified α-cells.

E. Incidence of Oscillation

Oscillation were calculated if the cells showed ≥ three times repetition of $[Ca^{2+}]_i$ increases after administration of Oxt.

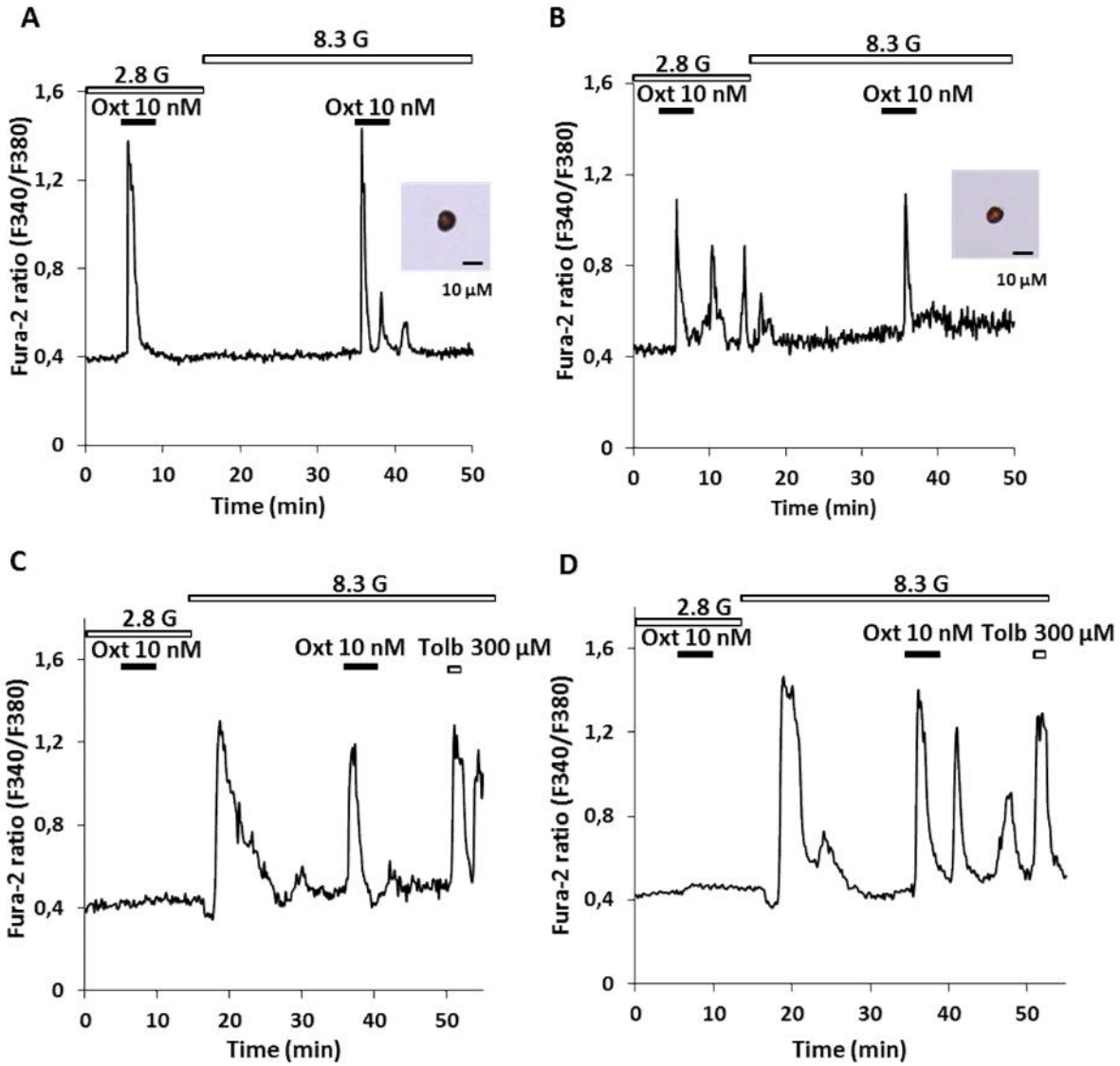

Fig. 1 Oxt induces $[Ca^{2+}]_i$ increases in α-cells and β-cells. Oxt induces $[Ca^{2+}]_i$ increases in α-cells in both 2.8 and 8.3 mM glucose in (A) single response and (B) oscillation response. In β-cells, Oxt induced $[Ca^{2+}]_i$ increases only at 8.3 mM glucose in (C) single response and (D) oscillation response

III. Results

A. Oxt Induces $[Ca^{2+}]_i$ Increases in α-Cells and β-Cells

Administration of Oxt (10 nM) induced increases in $[Ca^{2+}]_i$ in α-cells at both basal glucose concentration of 2.8 mM and stimulatory glucose concentration of 8.3 mM. Oxt induced $[Ca^{2+}]_i$ increases in an oscillatory pattern in 2 of 9 single α-cells at both 2.8 mM and 8.3 mM glucose (Figs. 1 (A) and (B)).

In β-cells, Oxt induced $[Ca^{2+}]_i$ increases only at 8.3 mM glucose but not at 2.8 mM glucose. Under 8.3 mM glucose Oxt induced $[Ca^{2+}]_i$ increases in an oscillatory pattern in 8 of 13 single β-cells (Figs. 1 (C) and (D)).

B. Oscillation Patterns Induced by Oxt on α-Cells and β-Cells

The peak amplitude of $[Ca^{2+}]_i$ increases in α-cells was not significantly different between 2.8 mM and 8.3 mM glucose. The number of cells which showed $[Ca^{2+}]_i$ oscillation after administration of Oxt was greater in β-cells than α-cells (Fig. 2 (C)). The AUC (area under the curve) of $[Ca^{2+}]_i$ increases in response to Oxt was not different between 2.8 mM glucose and 8.3 mM glucose in α-cells, and between α-cells and β-cells at 8.3 mM glucose.

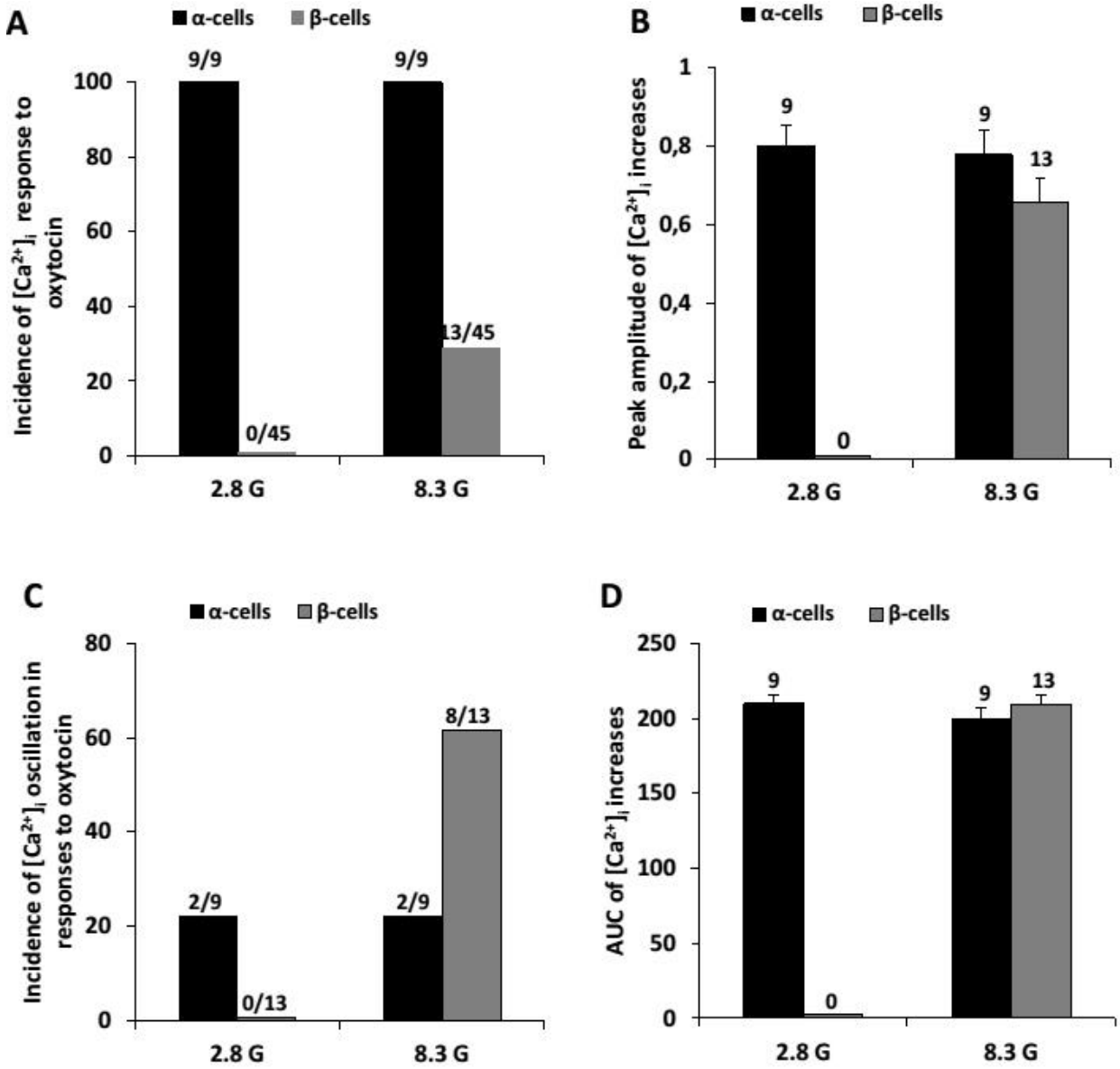

Fig. 2 Oscillation patterns induced by Oxt on α-cells and β-cells. (A) All α-cells responded to 10 nM Oxt at both 2.8 mM and 8.3 mM glucose concentration while in β-cells only at 8.3 mM glucose. (B) The peak amplitude of $[Ca^{2+}]_i$ increases in α-cells were not significantly different between 2.8 mM and 8.3 mM glucose. (C) The number of cells which showed oscillation after administration of Oxt was greater in β-cells than that of α-cells. (D) The area under the curve (AUC) of $[Ca^{2+}]_i$ increases in response to Oxt had no significant difference between 2.8 mM glucose and 8.3 mM glucose concentration in α-cells, and also was not significantly different when compared to AUC under 8.3 mM glucose concentration in β-cells

IV. Discussion

The present study demonstrated that both α-cells and β-cells respond to application of Oxt and show increase of $[Ca^{2+}]_i$. In α-cells, the $[Ca^{2+}]_i$ increase in response to Oxt was observed in both basal (2.8 mM) and stimulatory (8.3 mM) glucose concentration, whereas in β-cells, $[Ca^{2+}]_i$ increase induced by Oxt was observed only at stimulatory glucose concentration. This result may be reflecting the secretory patterns of α-cells and β-cells. In β-cells, insulin secretion occurs only in high glucose condition. However, in α-cells, glucagon secretion occurs in two peaks, low and high glucose concentration [20]. The patterns of its secretion and Oxt induced $[Ca^{2+}]_i$ increase were similar. Therefore, it is possible that Oxt potentiates the $[Ca^{2+}]_i$ increase when cells are under glucose concentration which promotes their contents to be released.

The fact that oscillation incidence was induced more frequently in β-cells compared to α-cells may also be explained by the glucose condition. Glucagon secretion from islets is high at low glucose concentration and suppressed by increasing glucose concentrations. Its maximal inhibition of glucagon secretion is observed around 7 mM glucose condition [21]. Under high glucose condition, insulin is released and then inhibited glucagon secretion [17]. However, 8.3 mM glucose is enough to induce maximum secretion of insulin from β-cells. Therefore, the fact that β-cells showed more oscillation of calcium increase in response to Oxt compared to that of α-cell may be reflecting the secretion capacity of its contents in each cell type.

In conclusion, the present study demonstrated that Oxt has a direct effect on α-cells and β-cells.

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