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Original Research

Oxytocin-stimulated insulin release in a clonal β-cell line RINm5F: involvement of phospholipase C-dependent and -independent pathways

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KEY WORDS islets of Langerhans; cultured cells; insulin; oxytocin; calcium; inositol 1,4,5-trisphosphate; U-73122; argipressin; phospholipase C

AIM: To study the mechanisms underlying oxytocin (Oxy)-induced insulin release. **METHODS:** *In a clonal pancreatic β-cell line, RINm5F cells.* **RESULTS:** Oxy increased insulin release and $[Ca^{2+}]_i$ in a concentration-dependent manner. Oxy-induced insulin release was not altered by pretreatment with pertussis toxin (PT). U-73122 ($2-8$ μmol·L^{-1}), a phospholipase C (PLC) inhibitor, concentration-dependently inhibited Oxy-induced increases in $[Ca^{2+}]_i$ with IC_{50} value of 2.8 ± 0.2 μmol·L^{-1}. In addition, U-73122 diminished the Oxy-induced increase in intracellular concentration of inositol 1, 4, 5-trisphosphate (IP_3). U-73122 at 8 μmol·L^{-1} totally abolished the Oxy-induced increases in $[Ca^{2+}]_i$ and IP_3; however it reduced the Oxy-induced increase in insulin release only by 36 % and 63 % in the monolayer and suspended cell preparations, respectively. **CONCLUSION:** Oxy increases insulin release through both PLC and non-PLC mediated signal transduction mechanisms.

Oxytocin (Oxy) stimulates myometrial contraction and milk ejection. Besides Oxy decreases glucose concentration in plasma[1] and stimulates insulin release[2]. The possibility that Oxy plays a role in the control of endocrine pancreas is supported by the presence of Oxy and its receptors in pancreatic islets[3].

Oxy activates a specific phospholipase C (PLC) which breaks down phosphoinositol 4,5-bisphosphate (PIP_2) to produce second messengers inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG) in mouse pancreatic islets[2], a hamster insulinoma cell line HIT-T15[4], and a rat insulinoma-cell line RINm5F[5]. IP_3 increases the release of Ca^{2+} from the endoplasmic reticulum to elevate $[Ca^{2+}]_i$, whereas DAG activates protein kinase C. However, the Oxy-induced insulin release in pancreatic islets is not accompanied by an increase in cAMP levels[2].

1-{6-[[17β-3-Methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl}-1*H*-pyrrole-2,5-dione (U-73122) is a specific PLC inhibitor in a number of cells including pancreatic β-cells[5]. In the present study, the mechanisms of Oxy-induced insulin release were studied.

MATERIALS AND METHODS

Materials Oxy, RPMI-1640 medium, and fetal bovine serum (FBS) were purchased from Sigma Chemical, USA; 24-well culture plates were purchased from Costar, MA, USA. Fura-2 was purchased from Molecular Probes, OR, USA; U-73122 was purchased from Biomol Res Lab, PA, USA. Porcine insulin was a gift of Eli Lilly Laboratories, IN, USA. The antiserum against porcine insulin was donated by Dr Joseph DUNBAR of Wayne State University, MI, USA.

Cell culture RINm5F cells were donated by Dr SB Pek (University of Michigan Medical Center, MI, USA). The cells were grown at 37 ℃ in 5 % CO_2 + 95 % air and maintained in RPMI-1640 medium with 10 % FBS[5]. All experiments were performed by using cells in passages 35 - 55.

Insulin release Static incubations were performed to study insulin release[6]. The cells were plated onto 24-well Costar plates at 10^5 cells/well and grown for 4 d. The growth medium was then removed, and insulin release from the RINm5F monolayer cells incubated with Krebs-Ringer bicarbonate (KRB) medium plus test agents was determined over a 5- or 15-min period at 37 ℃ after a 15-min preincubation. U-73122 was given 5 min before the administration of Oxy. Insulin content in the supernatants was measured by radioimmunoassay using rat insulin as

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standards.

Measurement of $[Ca^{2+}]_i$ $[Ca^{2+}]_i$ was measured by a Fura-2 fluorescence technique in RINm5F cell suspensions[17]. Briefly, RINm5F cells were resuspended in KRB buffer containing HEPES 10 mmol·L^{-1} glucose 4 mmol·L^{-1}, and 0.1 % bovine serum albumin. Fura-2 loading was performed by incubating the suspended cells with Fura-2 acetoxymethylester to a final concentration of 1 μmol·L^{-1} at 37 ℃ for 30 min. The fluorescence measurements were performed at room temperature by using a SLM8000 spectrofluorometer (SLM Instruments, IL, USA). Changes in the 340 nm − 380 nm fluorescence ratio were recorded.

Measurement of IP$_3$ IP_3 was measured by using a competitive radioreceptor-binding assay kit (DuPont Co, MA, USA). Cell suspensions 1.5×10^6 in 1 mL of KRB buffer were prepared in polypropylene tubes and incubated by shaking at 37 ℃. The incubation was terminated in 10 s by adding 20 % (w/v) ice-cold trichloroacetic acid. For the determination of the effect of U-73122 on PLC, Oxy was applied 100 s after the administration of U-73122.

Statistical analysis All values were expressed as $\bar{x} \pm s$. Results were analyzed by t-test for paired or unpaired values.

RESULTS

Effects of Oxy on insulin release, $[Ca^{2+}]_i$, and IP$_3$ Oxy (1 − 1000 nmol·L^{-1}) caused an increase in insulin release (Fig 1).

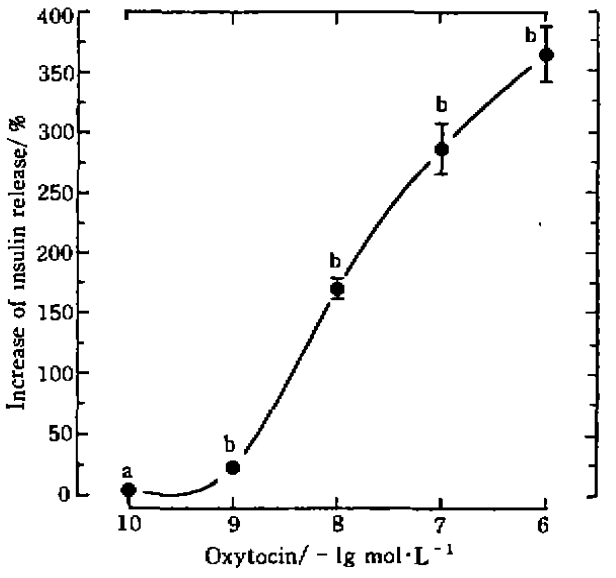

Fig 1. Effect of Oxy on insulin release in RINm5F cells. *Static incubation for 15 min was performed to measure* insulin release. $n = 4$, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$ *vs* control.

Oxy (10 − 1000 nmol·L^{-1}) increased $[Ca^{2+}]_i$ in a concentration-dependent manner (Fig 2).

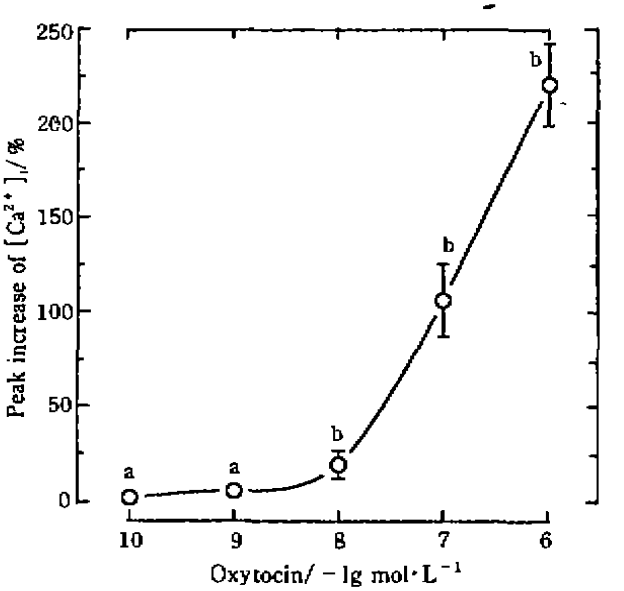

Fig 2. Effect of Oxy on $[Ca^{2+}]_i$ in RINm5F cells. $n = 4$, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$ *vs* control.

Oxy 1 nmol·L^{-1} did not significantly increase $[Ca^{2+}]_i$, but increased insulin release. Oxy 1 μmol·L^{-1} increased IP_3 production by >170 % of the basal level (Fig 3).

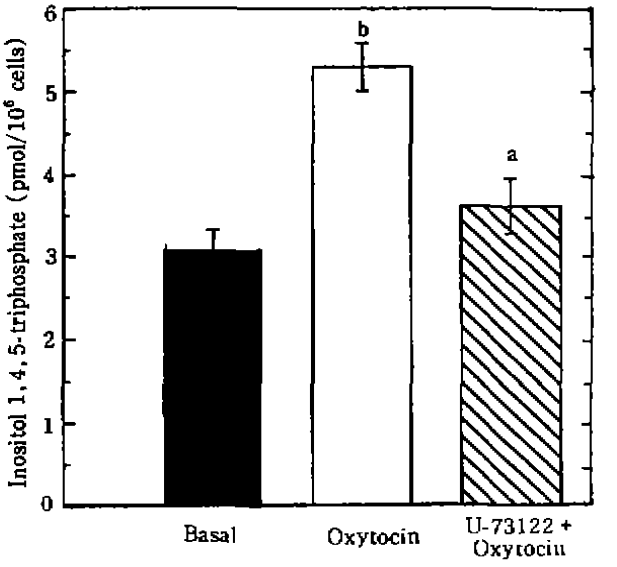

Fig 3. Inhibition by U-73122 of Oxy-induced production of IP$_3$ in RINm5F cells. U-73122 (8 μmol·L^{-1}) was given 100 s before Oxy (1 μmol·L^{-1}). The reaction was terminated after 10 s of Oxy application. $n = 4$, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$ *vs* basal control.

The rise in $[Ca^{2+}]_i$ after the administration of Oxy usually peaked within 30 – 40 s (the first phase) and decreased to a sustained level for the following 4 min (the second phase), which was seen in all concentrations of Oxy studied.

Effect of pertussis toxin (PT) and Oxy-induced $[Ca^{2+}]_i$ and insulin release Both Oxy (0.1 μmol·L^{-1})-induced increases in $[Ca^{2+}]_i$ and insulin release were not significantly altered by the overnight pretreatment with PT (0.1 mg·L^{-1}). Oxy-stimulated insulin release was 469 ± 26 pg·min^{-1} per 10^6 cells (n = 8) and Oxy-stimulated insulin release after overnight treatment with PT was 486 ± 23 pg·min^{-1} per 10^6 cells (n = 8). PT alone did not significantly change basal insulin release. Oxy-stimulated $[Ca^{2+}]_i$ was 216 ± 25 nmol·L^{-1} (n = 4) and Oxy-stimulated $[Ca^{2+}]_i$ after 2-h treatment with PT was 229 ± 18 nmol·L^{-1} (n = 4). PT alone did not significantly change basal Ca^{2+} concentrations.

Effect of U-73122 on Oxy-induced insulin release, $[Ca^{2+}]_i$, and IP_3 U-73122 (2 – 8 μmol·L^{-1}) inhibited Oxy (0.1 μmol·L^{-1})-induced insulin release in a concentration-dependent manner (Fig 4). But U-73122 alone did not change basal insulin release. At the highest concentration studied (8 μmol·L^{-1}), U-73122 inhibited Oxy-induced insulin release by 36 % and 63 % in the monolayer (Fig 4A) and suspended cell preparations (Fig 4B), respectively.

U-73122 alone did not change $[Ca^{2+}]_i$ except that at 8 μmol·L^{-1}, it increased $[Ca^{2+}]_i$ by 10 % of the basal level, which was subsided within 100 s of the administration. Pretreatment with U-73122 (1 – 8 μmol·L^{-1}) for 100 s concentration-dependently inhibited the Oxy (0.1 μmol·L^{-1})-induced increase in $[Ca^{2+}]_i$ (Fig 5). U-73122 at 8 μmol·L^{-1} abolished the Oxy (0.1 μmol·L^{-1})-induced increase in $[Ca^{2+}]_i$ (Fig 5) and production of IP_3 (Fig 3).

DISCUSSION

This study was undertaken to investigate the mechanisms by which Oxy increases insulin release in RINm5F cells. Oxy increased insulin release and $[Ca^{2+}]_i$ in a concentration-dependent manner.

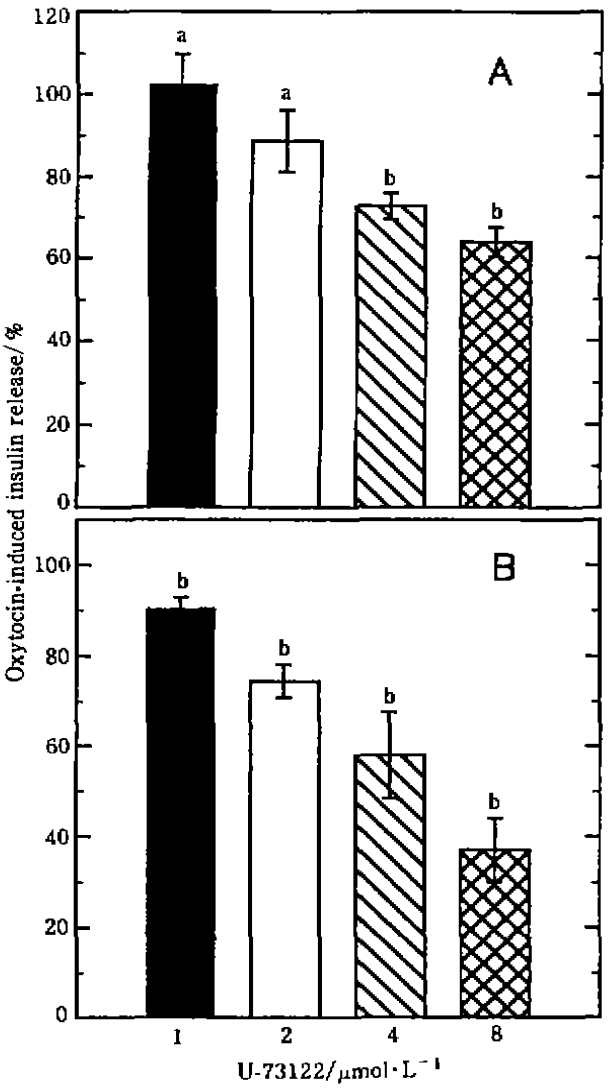

Fig 4. Effect of U-73122 on Oxy-induced insulin release in RINm5F cells. (A) Static incubations for 5 min. (B) Incubation of suspended cells for 5 min. U-73122 was given 100 s before Oxy (0.1 μmol·L^{-1}). n = 4, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$ vs Oxy-alone.

U-73122, a specific PLC inhibitor, inhibited Oxy-induced insulin release and $[Ca^{2+}]_i$. U-73122 at the highest concentration studied (8 μmol·L^{-1}) abolished Oxy-induced increase in $[Ca^{2+}]_i$ and IP_3, but U-73122 only reduced the Oxy-induced insulin release. Therefore, it is likely that Oxy stimulates insulin release through both PLC-dependent and -independent pathways.

Ca^{2+} plays a major role in stimulating insulin release[8]. Insulin release under physiological conditions is critically dependent on Ca^{2+} release

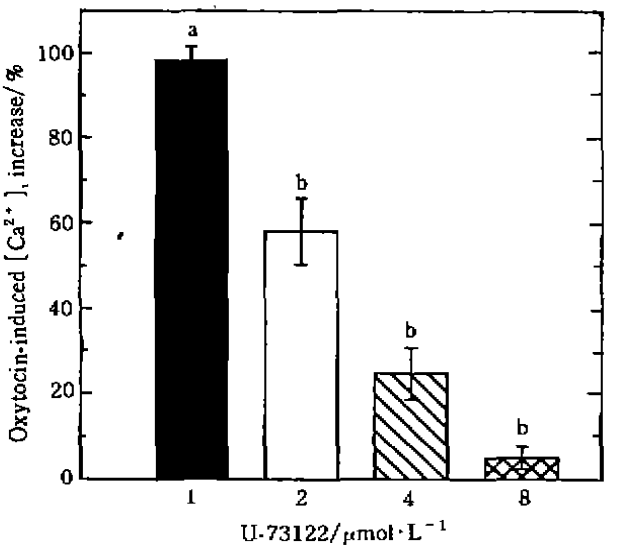

Fig 5. Effect of U-73122 on Oxy-induced increase in $[Ca^{2+}]_i$ at peak in RINm5F cells. U-73122 was given 100 s before Oxy (0.1 μmol·L^{-1}). $n = 4$, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$ *vs* Oxy-alone.

from internal Ca^{2+} stores and Ca^{2+} influx through the L-type voltage-dependent Ca^{2+} channels (VDCC)[9]. A number of hormones and neurotransmitters can induce insulin release by releasing Ca^{2+} into the cytosol. Argipressin (Arg), another neurohypophyseal hormone which shares high homology with Oxy by the differences of two amino acids, causes a biphasic rise in $[Ca^{2+}]_i$[5]. The initial $[Ca^{2+}]_i$ transient is due to the release from internal Ca^{2+} stores and the sustained phase of the increase in $[Ca^{2+}]_i$ is due to Ca^{2+} influx through channels including L-type VDCC[5,8]. Oxy also causes a biphasic increase in $[Ca^{2+}]_i$ through Ca^{2+} release and influx. The fact that nimodipine, an L-type VDCC blocker, only partially reduces the Ca^{2+} influx stimulated by Oxy, raises a possibility that other types of Ca^{2+} channels in addition to the L-type may be activated by Oxy (unpublished observation). Our findings are consistent with those of others[5,8] with regard to the Arg-induced increase in $[Ca^{2+}]_i$.

U-73122, a specific PLC inhibitor, to block the formation of IP_3 and DAG in many cell preparations[10], does not change the effect of ionomycin on the increase of $[Ca^{2+}]_i$ in RINm5F cells[5], even though ionomycin raises $[Ca^{2+}]_i$ predominantly by increasing Ca^{2+} release from the endoplasmic reticulum[11].

In the present study, U-73122 at the highest concentration studied (8 μmol·L^{-1}) abolished Oxy-induced increase in $[Ca^{2+}]_i$ and IP_3, but it only reduced the Oxy-induced insulin release. Thus, PLC-independent pathways may also contribute to the effect of Oxy on insulin release from RINm5F cells. The possible mechanisms by which Oxy increases insulin release may be through the activation of phospholipase A_2 (PLA_2) and phospholipase D (PLD). Arg-induced insulin release is not altered by a PLA_2 inhibitor, *N*-(*p*-amylcinnamoyl) anthranilic acid[5] and Oxy stimulates insulin release via V_{1b} Arg receptors[12,13]. Based on these findings, there is little possibility that Oxy stimulates insulin release by activating PLA_2. PLD induced-insulin release does not require Ca^{2+} influx or activation of PKC[14]. In fact, Oxy stimulates hydrolysis of phosphatidylcholine in HIT-T15 cells[4]. In addition, guanine nucleotides induce Ca^{2+}-independent insulin release by activating a site different from the transmembrane signalling systems[15]. The above are possible PLC-independent pathways for Oxy-induced insulin release.

In summary, Oxy increased insulin release, $[Ca^{2+}]_i$ and IP_3 in RINm5F cells. The effect of Oxy on $[Ca^{2+}]_i$ was antagonized by U-73122 in a concentration-dependent manner, but the effect of Oxy on insulin release was only reduced by U-73122. Our findings suggested that Oxy activates multiple signal transduction pathways to stimulate insulin release from RINm5F cells, possibly via both PLC-dependent and -independent pathways.

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缩宫素刺激克隆的 β-细胞系 RINm5F 内胰岛素释放：涉及磷脂酶 C 依赖性及非依赖性通道

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关键词 胰岛; 培养的细胞; 胰岛素; 缩宫素; 钙; 肌醇 1,4,5-三磷酸; U-73122; 精氨加压素; 磷脂酶 C

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