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Activin A increases intracellular free calcium concentrations in rat pancreatic islets

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Activin A stimulated insulin secretion in rat pancreatic islets, an effect that was attenuated by reduction of extracellular Ca^{2+} and abolished by either nitrendipine or verapamil. Activin A increased intracellular the free Ca^{2+} concentration, $[Ca^{2+}]_i$ in fura-2-loaded islets. Activin A-mediated elevation of $[Ca^{2+}]_i$ was abolished by the reduction of extracellular Ca^{2+} or the addition of nifedipine. In addition, activin A did not increase $[Ca^{2+}]_i$ in the presence of diazoxide, an opener of ATP-sensitive K^+ channels. These results suggest that activin A increases insulin secretion by stimulating Ca^{2+} entry.

Activin A; Calcium; Islet; Insulin secretion; Calcium influx; Calcium channel

1. INTRODUCTION

Activin A is a homodimeric protein with molecular weight of 25 kDa originally isolated from ovarian fluid as a stimulator of secretion of follicle-stimulating hormone [1]. The structure of activin A resembles, in many aspects, that of transforming growth factor-β (TGF-β), and it is now considered to be a member of the TGF-β supergene family. Accordingly, activin A elicits a variety of effects in many types of cells and, in particular, modifies cellular growth and differentiation as an autocrine or paracrine factor [2–6].

We reported previously that activin A augmented insulin secretion in rat pancreatic islets [7]. When studied in a perifusion system [8], it induced a biphasic secretory response of insulin in the presence of 2.8 mM glucose. Of interest is the a fact that immunoreactivity to activin A exists in pancreatic islets [8,9]. Immunoreactive activin A localizes mainly in secretory granules in α-cells. Furthermore, activin A and glucagon co-exist in the same secretory granules in α-cells and both may be released simultaneously. These results raise the possibility that activin A may play a physiological role in the regulation of insulin secretion.

Previous studies in our laboratory demonstrate that activin A affects cellular Ca^{2+} metabolism. For example, in erythroleukemia cells and in isolated hepatocytes, activin A increases intracellular free Ca^{2+} concentrations, $[Ca^{2+}]_i$, by causing breakdown of phosphoinositides [10,11]. In pituitary FSH secreting cells, activin A increases $[Ca^{2+}]_i$, but the effect is totally dependent on extracellular Ca^{2+} [12]. Since intracellular Ca^{2+} is a primary regulator of insulin secretion in β-cells of pancreatic islets [13], it is of interest to examine whether activin A affects cellular Ca^{2+} metabolism in β-cells. The present study was conducted to address this issue. The results indicate that activin A causes insulin secretion by stimulating Ca^{2+} entry.

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2. MATERIALS AND METHODS

2.1. *Preparation of islets and measurement of insulin secretion*

Pancreatic islets were isolated from male Wistar rats using collagenase according to Lacy and Kostianovski [14]. Islets were suspended in modified Kreb's–Ringer bicarbonate (KRB) buffer containing 20 mM HEPES/NaOH (pH 7.4), 5 mM $NaHCO_3$, 2.0 mM Ca^{2+}, 4.5 mM K^+ and 0.1% bovine serum albumin equilibrated with 100% O_2. Glucose concentration of the medium was 2.7 mM unless otherwise mentioned. Islets were incubated for 60 min and the supernatant was stored at −20°C for the measurement of insulin. Insulin was measured by radioimmunoassay using rat insulin as standard.

2.2. *Monitoring of intracellular free Ca^{2+} concentration*

Intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) was monitored by measuring fluorescence of fura-2. For loading of fura-2, islets were incubated in KRB buffer containing 2 μM fura-2/AM for 30 min at room temperature. After loading, islets were attached to a coverglass using Cell-tak, and were incubated in KRB buffer containing 50 μM probenecid to reduce leakage of fura-2 [15]. Fluorescence from a single islet was monitored by a microfluorometer as described previously [16]. The coverglass was mounted in a fluorescent microscope (Nikon, Tokyo, Japan) and was superfused with KRB buffer containing 50 μM probenecid at a flow rate of 1 ml/min. The temperature of the perifusate was 34°C. The islet was excited alternately with 340 and 380 nm at 60 Hz. Emissions (510 nm) from each excitation wavelength were collected, and average of the ratio of two emissions (340/380 nm) was calculated every second. The 340/380 ratio was monitored [16] and was not calibrated in terms of free Ca^{2+} concentration since the distribution of fura-2 in the islet was not known.

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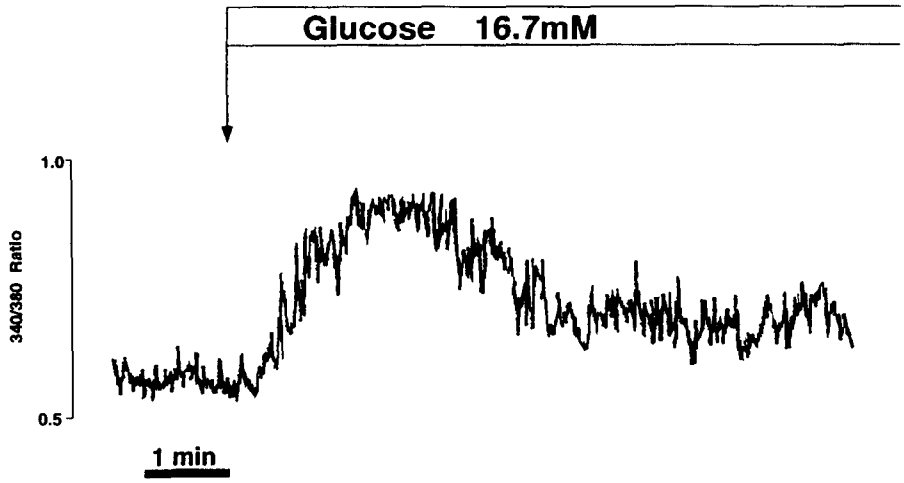


Fig. 1. Effect of glucose on intracellular free Ca^{2+} concentration. A fura-2-loaded islet was stimulated with 16.7 mM glucose and the 340/380 nm ratio was monitored. The result is the representative of ten experiments.

2.3. *Measurement of cAMP*

Islets were incubated for 15 min in KRB buffer containing 0.5 mM 3-isobutyl-1-methylxanthine and various stimulators. Trichloroacetic acid was then added to terminate the reaction. After the removal of trichloroacetic acid by washing with diethylether, cAMP was measured after succinylation using a radioimmunoassay kit (Yamasa, Tokyo, Japan).

3. RESULTS

3.1. *Effect of reduction of extracellular Ca^{2+} on activin A-induced insulin secretion*

It is well known that Ca^{2+} plays an essential role in the regulation of insulin secretion [13]. We therefore examined whether changes in extracellular Ca^{2+} would affect activin-mediated insulin secretion in a batch incubation system. In the presence of 2.0 mM extracellular Ca^{2+}, 10 nM activin A induced an approximately 3-fold increase in insulin secretion. Reduction of extracellular Ca^{2+} attenuated both basal and activin-mediated insulin secretion (Table I). When the extracellular Ca^{2+} concentration was 100 μM or less, activin A did not augment insulin secretion.

3.2. *Effect of Ca^{2+} channel blockers on activin-mediated insulin secretion*

In the next set of experiments, we examined the effect of Ca^{2+} channel blockers on activin-mediated insulin secretion. As shown in Table II, nifedipine, a dihydropyridine antagonist of L-type voltage-dependent Ca^{2+} channels, completely blocked activin-induced insulin secretion. Similarly, verapamil completely inhibited the action of activin A in islets.

3.3. *Effect of activin A on intracellular free Ca^{2+} concentration*

We monitored changes in $[Ca^{2+}]_i$ by measuring fura-2 fluorescence in a single islet. Elevation of ambient glucose to 16.7 mM resulted in an elevation of $[Ca^{2+}]_i$ after about a 20 s lag period. The initial rise in $[Ca^{2+}]_i$ was followed by a sustained plateau phase (Fig. 1). When 10

Table I

Effect of reduction of extracellular Ca^{2+} on activin A-mediated insulin secretion

Extracellular calcium (mM)	Insulin (μU/h/islet)	
	None	Activin A
2.0	29.4 ± 6.3	72.4 ± 12.1
1.0	25.3 ± 7.1	64.2 ± 9.6
0.5	16.3 ± 5.2	23.2 ± 6.2
0.1	6.3 ± 2.4	7.4 ± 2.8
0.05	2.1 ± 1.2	2.5 ± 1.0

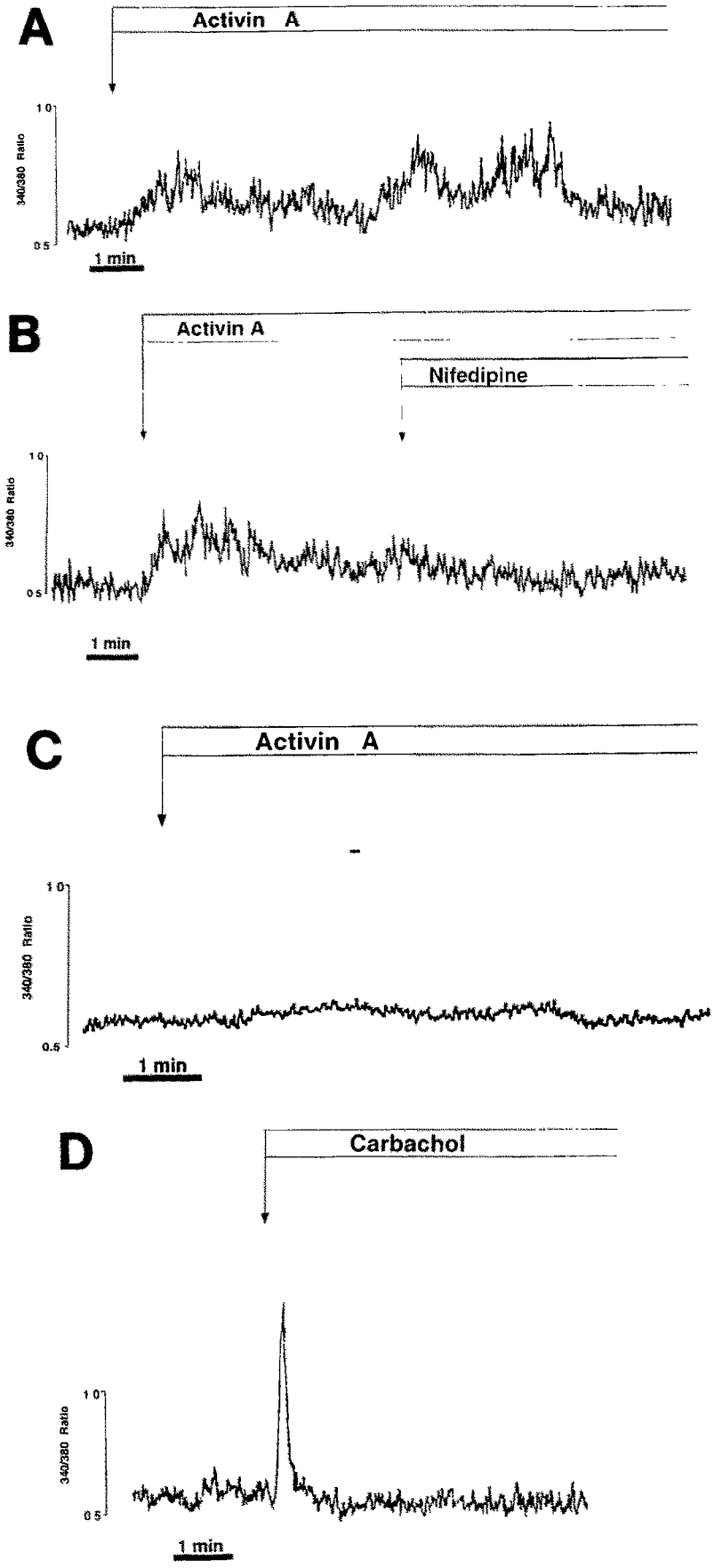
Islets were incubated for 60 min with or without 10 nM activin A in the presence of varying concentrations of extracellular Ca^{2+}. Values are the mean ± S.E.M. for five experiments.

Table II

Effect of Ca^{2+} channel blocker on activin A-induced insulin secretion

Addition	Insulin (μU/h/islet)
None	34.1 ± 4.3
+nifedipine	31.3 ± 6.2
+verapamil	31.8 ± 5.3
10 nM activin A	66.3 ± 7.3*
+nifedipine	36.4 ± 9.6**
+verapamil	39.3 ± 8.1**

Islets were incubated for 60 min with or without 10 nM activin A in the presence and absence of either 1 μM nifedipine or 10 μM verapamil. Values are the mean ± S.E.M. for four experiments. Concentration of extracellular Ca^{2+} was 2.0 mM. Statistical analysis was done by Student's *t*-test. *$P < 0.01$ vs. none, **$P < 0.01$ vs. 10 nM activin A.


A
Activin A
1.0
340/380 Ratio
0.5
1 min
B
Activin A
Nifedipine
1.0
340/380 Ratio
0.5
1 min
C
Activin A
1.0
340/380 Ratio
0.5
1 min
D
Carbachol
1.0
340/380 Ratio
0.5
1 min

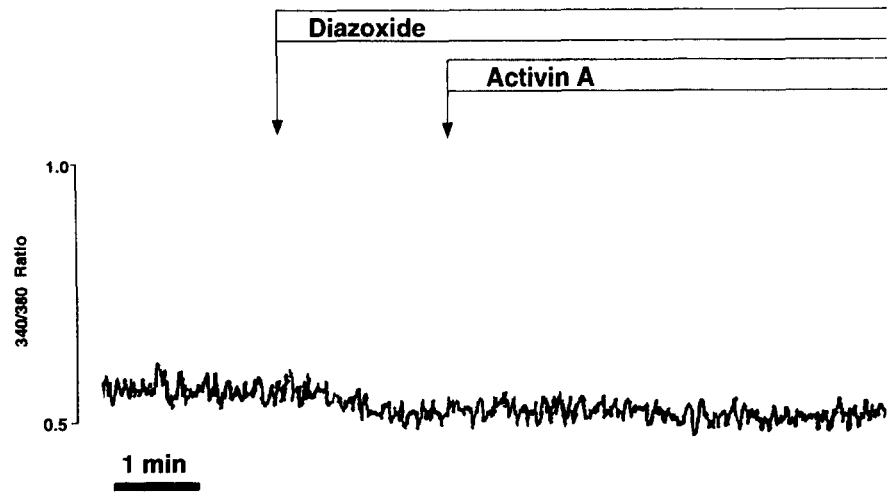


Fig. 3. Effect of activin A in the presence of diazoxide. A fura-2-loaded islet was stimulated by 10 nM activin A in the presence of 100 μM diazoxide.

nM activin A was added to an islet, a sustained elevation of $[Ca^{2+}]_i$ was observed. The elevation was detected without a lag period, and burst-like elevation of $[Ca^{2+}]_i$ was superimposed on to the sustained phase (Fig. 2A). Addition of 1 μM nifedipine to activin A-stimulated islets abolished the burst-like elevation of $[Ca^{2+}]_i$ (Fig. 2B). Activin A did not cause any change in $[Ca^{2+}]_i$ in the presence of 10 μM extracellular Ca^{2+} (Fig. 2C). Under similar conditions, 10 μM carbachol induced an immediate increase in $[Ca^{2+}]_i$ (Fig. 2D). When the plasma membrane of the β-cells was hyperpolarized by adding 100 μM diazoxide, an opener of ATP-sensitive K^+ channels, $[Ca^{2+}]_i$ was decreased slightly: under these conditions, activin A did not cause any change in $[Ca^{2+}]_i$ (Fig. 3).

3.4. *Effect of activin A on cAMP*

As shown in Table III, 10 nM glucagon induced a marked increase in cAMP in islets in the presence of 3-isobutyl-1-methylxanthine. Under similar conditions, activin A did not affect cellular cAMP.

4. DISCUSSION

The present results demonstrate for the first time that activin A increases $[Ca^{2+}]_i$ in rat pancreatic islets. Although we monitored $[Ca^{2+}]_i$ in a single islet, which contains multiple types of cells, the elevation of $[Ca^{2+}]_i$ presumably reflects $[Ca^{2+}]_i$ in β-cells for the following reasons. First, β-cells are the most abundant cells in the islet and $[Ca^{2+}]_i$ response to high glucose, which occurs in β-cells, is in fact detected in our system. Second, the increase in $[Ca^{2+}]_i$ in response to activin A is accompanied by an enhancement of insulin secretion. Conversely, insulin secretion is attenuated when activin-mediated elevation of $[Ca^{2+}]_i$ is blocked by nitrendipine. Thirdly, diazoxide, which activates ATP-sensitive K^+ channel in β-cells, reverses the action of activin A on $[Ca^{2+}]_i$

The present results indicate that activin A appears to stimulate insulin secretion by increasing $[Ca^{2+}]_i$ in β-cells. The action of activin A is completely blocked by an addition of a dihydropyridine Ca^{2+} channel blocker, nifedipine, or by reducing extracellular Ca^{2+}. This is in contrast to the action of carbachol, which causes breakdown of phosphoinositide [17] and increases $[Ca^{2+}]_i$ mainly by causing release of Ca^{2+} from intracellular pools. It is therefore unlikely that activin A increases $[Ca^{2+}]_i$ by causing phosphoinositide turnover. Presumably, activin A may increase $[Ca^{2+}]_i$ by stimulating Ca^{2+} entry via voltage-dependent Ca^{2+} channels. Consistent with this idea is the a fact that diazoxide, which hyperpolarizes the plasma membrane, attenuates the action of activin A on $[Ca^{2+}]_i$. It should be mentioned, however, that our results do not address the involvement of ATP-sensitive K^+ channels in the action of activin A. It is reported that agents which increase cAMP elevate $[Ca^{2+}]_i$ by stimulating Ca^{2+} influx [18], yet, as shown in

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Fig. 2. Effect of activin A on intracellular free Ca^{2+} concentration. (A,B) A fura-2-loaded islet was stimulated with 10 nM activin A, and 1 μM nifedipine was added as indicated (B). (C,D) A fura-2-loaded islet was stimulated by 10 nM activin A (C) or 10 μM carbachol (D) in the presence of 10 μM extracellular Ca^{2+}. The results are representative of at least three experiments with similar results.

Table III

Effect of activin A and glucagon on cAMP

Addition	cAMP (pmol/30 min/islet)
None	0.45 ± 0.15
Activin A	0.51 ± 0.20
Glucagon	5.81 ± 1.20

Islets were incubated for 30 min with either 10 nM activin A or 10 nM glucagon and cAMP was measured. Values are the mean ± S.E.M. for five experiments.

Table III, activin A does not augment production of cAMP. Taken together, the effect of activin A on islet Ca^{2+} metabolism is quite unique in that it stimulates Ca^{2+} entry by a mechanism independent of cAMP.

Since the action of activin A is blocked by dihydropyridine, it is likely that activin A activates, either directly or indirectly, voltage-dependent Ca^{2+} channels. We have reported recently that activin A increases $[Ca^{2+}]_i$ by stimulating Ca^{2+} influx in FSH-secreting pituitary tumor cells [12]. In these cells, activin A stimulates Ca^{2+} entry by causing depolarization and thereby activating voltage-dependent Ca^{2+} channels [19]. It is tempting to speculate that activin A stimulates Ca^{2+} entry by changing the membrane potential in pancreatic β-cells. Further study is required to elucidate the mechanism of action of activin A in islet β-cells.

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