Vol. 156, No. 1, 1988 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 14, 1988 Pages 335-339

A NOVEL ACTION OF ACTIVIN A: STIMULATION OF INSULIN SECRETION IN RAT PANCREATIC ISLETS

Yasuo Totsuka, Mari Tabuchi, Itaru Kojima,
Hiroshiro Shibai* and Etsuro Ogata

Fourth Department of Internal Medicine
University of Tokyo School of Medicine
Tokyo, Japan
*Central Research Laboratory, Ajinomoto,CO.
Kawasaki, Japan

Received August 26, 1988

The present study was conducted to examine an action of activin A on insulin secretion from rat pancreatic islets. In a batch incubation system, activin A stimulated insulin secretion in a dose-dependent manner at concentrations higher than 1 nM. Furthermore, activin A greatly potentiated glucose-induced insulin release. When islets were perifused with 1 nM activin A, insulin secretion was barely affected in this system. However, the insulin response to 16.7 mM glucose was greatly enhanced. Both the first and second phases of insulin response were enhanced by 1 nM activin A. These results indicate that, in addition to its known actions on pituitary-gonadal and hematopoietic systems, activin A modulates the function of pancreatic islets and stimulates insulin secretion.

Activin A is a polypeptide with a molecular weight of 25,000 Da purified from ovarian fluid as a stimulator of follicle-stimulating hormone (FSH) secretion (1). It is a homodimer of the β -subunit of inhibin A, an inhibitory peptide of FSH secretion isolated from ovarian fluid (2). A related polypeptide activin, which is a heterodimer of β -subunits of inhibin A and inhibin B (3), also increases FSH secretion (see ref. 4 for review).

Address correspondence to: Etsuro Ogata, M.D., Ph.D.; Fourth Department of Medicine; University of Tokyo School of Medicine; 3-28-6 Mejirodai; Bunkyo-ku; Tokyo 112, Japan

0006-291X/88 $1.50

Independent of these studies, Eto et al. isolated a polypeptide which causes differentiation of Friend erythroleukemia cells from conditioned medium of a phorbol ester-treated human monocytic leukemia cell line (5). The primary structure of this polypeptide, erythroid differentiation factor, is identical to that of activin A (6) and was shown to stimulate FSH secretion from primary culture of rat pituitary cells (7). Since activin A acts on at least pituitary, gonadal and erythroid cells (5), and since mRNA for activin A is found in several tissues (8), it is possible that activin A modulates various cell functions as a local regulator or a cytokine.

In the present study, we examined the effect of activin A on insulin secretion in rat pancreatic islets. The results indicate that activin A is a potent stimulator of insulin secretion.

Materials and Methods

Pancreatic islets were isolated from male Wistar rats weighing 200 g by using collagenase as described by Lacy et al. (9,10). Islets were suspended in a solution containing 115 mM NaCl, 5 mM KCl, 2.2 mM $CaCl_2$, 1 mM $MgCl_2$, 24 mM $NaHCO_3$, and 2.8 mM glucose equilibrated with 95% O_2 and 5% CO_2 gas. For batch incubation, an islet was incubated in 1 ml medium for 120 min at 37 ° C. Perifusion of islets was performed as described by Zawalich et al. (11). Insulin was measured by radioimmunoassay using rat insulin (Novo Research Institute, Copenhagen, Denmark) as standard. Activin A was purified as described previously (5).

Results

To determine the effect of activin A on insulin secretion in pancreatic islets, we incubated islet with varying concentrations of activin A in the presence of a nonstimulatory concentration of glucose. As depicted in Figure 1, activin A increased insulin secretion at concentrations higher than 1 nM. Activin A-induced insulin release increased in a concentration-dependent manner and was almost saturated at 10 nM.

Figure 2 demonstrates the effect of activin A on glucose-mediated insulin secretion. In the absence of activin A, 16.7 mM glucose induced approximately 14-fold stimulation of insulin release. In the presence of 10

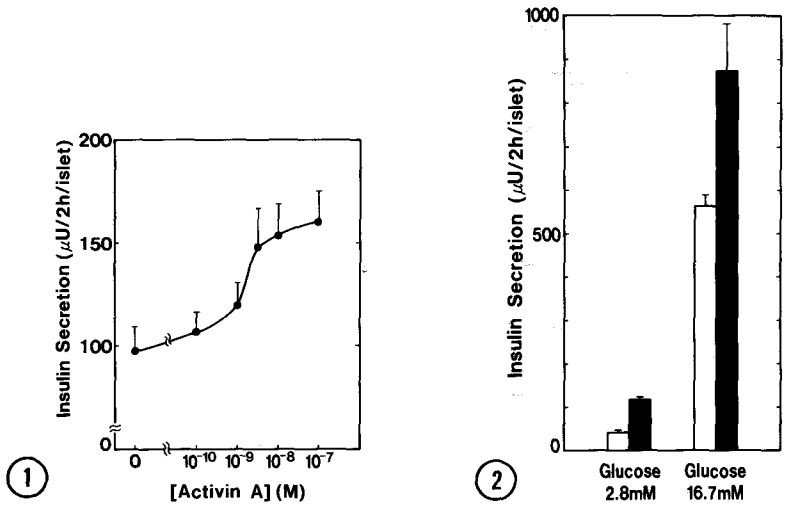

Figure 1. Dose Response Curve for Activin A-induced Insulin Secretion

An islet was incubated with various concentrations of activin A in the presence of 2.8 mM glucose for 120 min. Data are the mean ± S.E. for six determinations and the representative of three experiments with similar results.

Figure 2. Effect of Activin A on Glucose-induced Insulin Secretion

An islet was incubated with either 2.8 or 16.7 mM glucose in the presence and absence of 10 nM activin A for 120 min. Data are the mean ± S.E. for five determinations and the representative of three experiments with similar results.

nM activin A, glucose-mediated insulin secretion was greatly potentiated. The action of activin A was more than additive.

In the next set of experiments, we examined the effect of activin A on insulin secretion in perifusion system. As reported previously, 16.7 mM glucose induced a biphasic increase in insulin release. When 1 nM activin A was added in the presence of 2.8 mM glucose, it did not stimulate insulin secretion significantly. In the presence of 1 nM activin A , however, the insulin response to 16.7 mM glucose was greatly enhanced. The enhancement was evident in both first and second phases of insulin response. In particular, the second phase of glucose-mediated secretion was approximately 200% of that in the absence of activin A (Figure 3). It should be mentioned that the action of activin A was greater in the perifusion system than in the static incubation system.

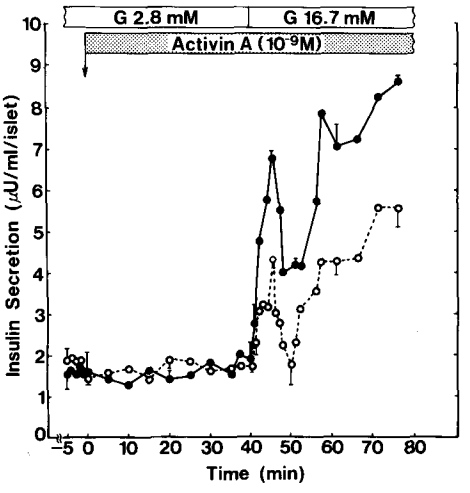

Figure 3. Effect of Activin A on Glucose-induced Insulin Secretion in Perifusion System

Islets were stimulated by 16.7 mM glucose as indicated in the presence (●) and absence (○) of 1 nM activin A . Values are the mean ± S.E. for three experiments.

Discussion

Results obtained in the present study clearly indicate that activin A acts on rat pancreatic islets to stimulate insulin secretion. Although concentrations of activin A which increase insulin secretion are relatively high, the results in Figure 3 demonstrate that a relatively low concentration of activin A (1 nM) enhances glucose-mediated insulin secretion. It should be emphasized that the action of activin A and glucose are synergic rather than additive. Hence, activin A potentiates glucose-mediated secretion. The mode of action of activin A resembles, in some aspect, that of cholecystokinin (CCK), which by itself is a weak stimulator but greatly potentiates glucose-mediated secretion (12). At present the mechanism by which activin A potentiates glucose action is not known. It is tempting to speculate that activin A causes phosphoinositide turnover in β -cells since activin A causes hydrolysis of phosphoinositide in Friend erythroleukemia cells (13) and since CCK potentiates glucose action by causing phosphoinositide turnover (14). It is possible that, in addition to phosphoinositide turnover, activin A may activate an additional transduction mechanism.

The present results are in agreement with our unpublished observation that intraperitoneal administration of activin A results in a marked decrease in plasma glucose concentration in mice. It is quite likely that activin A modulates insulin secretion both in vivo and in vitro. At present the physiological significance of activin A-induced insulin secretion is not certain. It is presumed that both inhibin and activins are present in plasma (4). It is thus possible that activin A in plasma affects insulin secretion in vivo. More importantly, it is also possible that activin A exists in pancreatic islets and modulates insulin secretion as a local regulator. Studies are now in progress in our laboratory to examine this possibility.

Acknowledgment

This work was supported by grants from The Ministry of Education, Science and Culture of Japan and Workshop on Cell Calcium Signal in Cardiovascular System.

References

1) Vale,W.,Rivier,J.,Vaughan,J.,McClintock,R.,CorriganmA.,Woo,W.,Karr,D. and Spiess,J. (1986) Nature 321,776-778.
2) Ling,N.,Ying,S-Y.,Ueno,N.,Esch,F.,Denoroy,L. and Guillmin,R. (1985) Proc.Natl.Acad.Sci.USA. 82,7217-7222.
3) Ling,N.,Ying,S-Y.,Ueno,N.,Shimasaki,S.,Esch,F.,Hotta,M. and Guillmin,R. (1986) Nature 321, 779-781.
4) Ying,S-Y. (1988) Endocrine Review 9, 267-293.
5) Eto,Y.,Tsuji,T.,Takegawa,M.,Takano,S.,Yakagawa,Y. and Shibai,H. (1987) Biochem.Biophys.Res.Commun. 142, 1095-1099.
6) Murata,M.,Eto,Y.,Shibai,H.,Sakai,M. and Muramatsu,M. (1988) Proc.Natl.Acad.Sci.USA. 85,2434-2438.
7) Kitaoka,M.,Yamashita,N.,Eto,Y.,Shibai,H. and Ogata,E. (1987) Biochem. Biophys.res.Commun. 146,1382-1386.
8) Yu,J.,Shao,L.,Lemas,V.,Yu,A.L.,Vaughan,J.,Rivier,J. and Vale,W. (1987) Nature 330, 765-767.
9) Lacy,P.E. and Kostianovski,M., (1967) Diabetes 16, 35-39.
10) Sharp,D.W.,Kemp,C.B.,Knight,M.J.,Ballinger,W.F. and Lacy,P.E. (1973) Transplantation 16, 686-689.
11) Zawalich,W.,Brown,C. and Rasmussen,H. (1983) Biochem.Biophys.Res. Commun. 117,448-455.
12) Zawalich,W. and Daiz,V.A. (1987) Diabetes 36, 118-122.
13) Shibata,H.,Ogata,E.,Eto,Y.,Shibai,H. and Kojima,I. (1987) Biochem.Biophys.Res.Commun. 146,187-193.
14) Zawalich,W.,Daiz,V.A. and Zawalich,K.C. (1987) Diabetes 36, 1420-1424.