EFFECTS OF SOMATOSTATIN ON INSULIN RELEASE FROM DIGITONIN-PERMEABILIZED ISLETS

TATSUO TAMAGAWA¹, ICHIRO NIKI¹, KAZUMASA UEMURA¹, HISAYUKI MIURA¹, AKIHISA IGUCHI¹, HATSUMI NIKI² and ATSUSHI NIKI²

¹Third Department of Internal Medicine, Nagoya University School of Medicine, Showaku, Nagoya 466, and ²Department of Internal Medicine, Aichigakuin University School of Dentistry, Chikusaku, Nagoya 464, Japan

ABSTRACT

Somatostatin is known to be a powerful inhibitor of insulin release, but its cellular mechanism has not been established. We studied the effects of somatostatin on insulin release from digitonin-permeabilized rat pancreatic islets. A rise in a free Ca^{2+} concentration in the medium from 0.1 μM to 1–10 μM induced insulin release in a concentration-dependent manner when the islets had been treated with digitonin. The Ca^{2+} -induced release was not inhibited by somatostatin. Forskolin, an activator of adenylate cyclase, cAMP and 12-*O*-tetradecanoylphorbol-13-acetate, an activator of protein kinase C, augmented insulin release induced by 1 μM Ca^{2+} . Somatostatin inhibited the release augmented by these substances. The findings indicate that somatostatin differently affects insulin release mediated by the three major intracellular messenger systems.

Somatostatin (1, 30) inhibits insulin release caused by various stimuli. However, the cellular mechanism of the inhibitory action remains undefined. Because of the importance of Ca^{2+} as an intracellular messenger for hormone release, studies on Ca^{2+} flux have been extensively carried out to elucidate the inhibitory action of somatostatin. Several reports have suggested that somatostatin affects Ca^{2+} flux (6, 15, 16), but the other is contradictory to this view (28). Using a fluorescent Ca^{2+} indicator, it has recently been shown that somatostatin lowers cytosolic free Ca^{2+} concentration ($[\text{Ca}^{2+}]$) (21) and that the effect is transient (13).

Another approach to analyze the relationship between cytosolic $[\text{Ca}^{2+}]$ and insulin release is to clamp cytosolic $[\text{Ca}^{2+}]$ at arbitrary levels. For this purpose we used digitonin-permeabilized islets and found that catecholamines, powerful inhibitors of insulin release, might act on a step(s) subsequent to a rise in cytosolic $[\text{Ca}^{2+}]$ in stimulus-secretion coupling (23).

In the present study, we studied effects of somatostatin on insulin release from the Ca^{2+} -clamped islets.

MATERIALS AND METHODS

Islets were isolated from pancreata of male Wistar rats (250–350 g) by collagenase digestion, followed by preincubation for 1 h in Krebs-Ringer bicarbonate buffer containing 3 mM glucose and 5 mg/ml bovine serum albumin. A pH of 7.4 was maintained by equilibration with O_2/CO_2 (19:1, v/v). In experiments using intact islets, groups of 5 islets were incubated at 37°C for 30 min in the presence of 10 mM glucose with or without somatostatin (Sigma, St. Louis, MO). In other series of experiments, islets were treated with 20 μM digitonin (Sigma) for 5 min (22). After washing 3 times, groups of 5 islets were incubated at 37°C for 30 min in media containing various concentrations of Ca^{2+} and test substances. The medium used for digitonin treatment, washing, and incubation of the treated islets was the gassed (100% O_2) Ca-EGTA buffer with the following composition (mM): K-glutamate, 100; Na-glutamate, 42; Mg-ATP, 3; Hepes, 16; EGTA, 1; and bovine serum albumin (5 mg/ml). No glucose was added. $[\text{Ca}^{2+}]$ in the buffer was adjusted by adding appropriate concentrations of CaCl_2 , based on the calculation using the

Fig. 1 Effect of somatostatin on glucose-induced insulin release from intact islets. Groups of 5 islets were incubated for 30 min at 37°C in the medium containing 10 mM glucose and indicated concentrations of somatostatin. Values are means $\pm$ SE (represented by bars), for 5–7 observations from 1 experiment. Significance levels: $0.01 < *P \leq 0.05$, $**P \leq 0.01$ vs the value with 10 mM glucose alone

stability constant of EGTA (7). The pH was adjusted to 7.0 with NaOH. 12-*O*-Tetradecanoylphorbol-13-acetate (TPA) (Sigma) was dissolved in dimethyl sulfoxide, and forskolin (California, La Jolla, CA) in 95% ethanol to obtain stock solutions of 162 μ M and 20 mM, respectively. Inositol 1,4,5-trisphosphate (IP_3) was purchased from Sigma and cAMP from Boehringer Mannheim, Germany. Insulin content in the medium was determined by radioimmunoassay using Insulin-Riabeed (Dainabot, Tokyo). Data are given as means $\pm$ SE and statistical significance was assessed by analysis of variance with Duncan's multiple range test or Williams-Wilcoxon's test.

RESULTS

Effects of Somatostatin on Insulin Release from Intact Islets

Somatostatin was tested at concentrations ranging from 1 ng/ml to 10 μ g/ml (0.61 nM to 6.1 μ M) in the presence of 10 mM glucose. Somatostatin higher than 10 ng/ml inhibited glucose-induced insulin release dose-dependently (Fig. 1). Maximal inhibi-

tion of insulin release from intact islets was seen with somatostatin at 1 μ g/ml; this concentration was used in experiments with Ca^{2+} -clamped islets.

Effects of Somatostatin on Insulin Release from Ca^{2+} -Clamped Islets

Increasing Ca^{2+} concentrations in the medium from 0.1 μ M to 1–10 μ M resulted in a concentration-dependent increase in insulin release from Ca^{2+} -clamped islets (Fig. 2). IP_3 (5 μ M) did not influence the release at any concentrations of Ca^{2+} tested (0.1–10 μ M) (data not shown). Somatostatin did not inhibit Ca^{2+} -induced release at 1 μ g/ml, nor even at 10 μ g/ml (data not shown). TPA and other substances which increase intracellular cAMP level have been shown to potentiate Ca^{2+} -induced insulin release from Ca^{2+} -clamped islets (22, 23). It thus seems interesting to examine the effect of somatostatin on insulin release potentiated by these substances. Addition of forskolin (5 μ M) into the medium resulted in the potentiation of the release at 1 μ M Ca^{2+} , which was antagonized by somatostatin dose-dependently (Fig. 3). The effec-

Fig. 2 Effects of somatostatin on insulin release from Ca²⁺-clamped islets at various concentrations of Ca²⁺. Isolated islets were treated with digitonin as described in the text. Groups of 5 treated islets were incubated for 30 min at 37°C in the medium containing indicated concentrations of Ca²⁺, and somatostatin. Values are means ± SE (represented by bars), for 7 observations from 1 experiment (0.1 µM Ca²⁺) and 17-18 observations from 3 independent experiments (1 and 10 µM Ca²⁺).

Fig. 3 Effects of somatostatin on forskolin-potentiated insulin release from Ca²⁺-clamped islets. Isolated islets were treated with digitonin as described in the text. Groups of 5 treated islets were incubated for 30 min at 37°C in the medium containing 1 µM Ca²⁺ and indicated concentrations of somatostatin with or without 5 µM forskolin. Insulin release is expressed in per cent of the value found in paired groups of islets incubated in the presence of 1 µM Ca²⁺ alone. Values are means ± SE (represented by bars), for 25-29 observations from 5 independent experiments. Significance levels: ***P* ≤ 0.01 vs 5 µM forskolin alone

Fig. 4 Effects of somatostatin on cAMP-potentiated insulin release from Ca^{2+} -clamped islets. Islets were treated with digitonin as described in the text. Groups of 5 islets were incubated for 30 min at 37°C in the medium containing 1 μM Ca^{2+} and indicated concentrations of cAMP with or without somatostatin (1 μg/ml). Values are means $\pm$ SE (represented by bars), for 22 observations from 3 independent experiments (0, 10 μM cAMP) and 12 observations from 2 independent experiments (5 mM cAMP). Significance levels: ** $P \leq 0.01$ between indicated columns, ns, not significant

tive concentration of somatostatin was similar to that found with intact islets. At 1 μM Ca^{2+} , somatostatin inhibited insulin release potentiated by 10 μM cAMP, but not that potentiated by 5 mM cAMP (Fig. 4). The effect of somatostatin on insulin release potentiated by TPA was compared with that potentiated by forskolin (Fig. 5). TPA increased insulin release by 360% and forskolin by 110%. Somatostatin inhibited the release to a similar extent (TPA, 39% and forskolin, 36%).

DISCUSSION

In the present study, islets were treated with digitonin and incubated in Ca-EGTA buffer. As discussed previously (22), cytosolic $[\text{Ca}^{2+}]$ of these islets is considered to be fixed by the buffer. It may be argued that $[\text{Ca}^{2+}]$ is increased transiently and/or locally even in Ca-EGTA buffer when intracellular stored Ca is mobilized in response to stimulation. One of the second messengers, which is known to mobilize stored Ca, is IP_3 . This substance

was reported to increase Ca^{2+} efflux from pre-labeled digitonin-treated islets (27) or cytosolic $[\text{Ca}^{2+}]$ in digitonin-treated RINm5F cells (3). We tested its effect on insulin release from permeabilized islets and found that the release was not affected. This suggests that Ca mobilized by IP_3 was completely chelated by EGTA, or that even if the chelation was not complete, such a small increase in $[\text{Ca}^{2+}]$ did not produce any significant insulin release under the present condition. Thus, it is likely that $[\text{Ca}^{2+}]$ is virtually clamped at arbitrary levels even on stimulation of this preparation, and that processes dependent on changes in $[\text{Ca}^{2+}]$ can be dissociated from those independent of changes in $[\text{Ca}^{2+}]$. The range of $[\text{Ca}^{2+}]$ where insulin release was stimulated in our study was higher than 2×10^{-7} M which was observed in experiments using a fluorescent Ca^{2+} indicator, quin2 (18, 29). One possibility may be that the permeabilized islets lose some amplifying factors for Ca^{2+} -induced release, as suggested in adrenal chromaffin cells (19). Alternatively, a high cytosolic $[\text{Ca}^{2+}]$ may be

Fig. 5 Effects of somatostatin on insulin release potentiated by forskolin and TPA from Ca^{2+} -clamped islets. Groups of 5 digitonin-treated islets were incubated for 30 min at 37°C in the medium containing $1 \mu\text{M}$ Ca^{2+} and indicated concentrations of test substances. Values are means $\pm$ SE (represented by bars), for 17 observations from 3 independent experiments. Significance levels: $**P \leq 0.01$ between indicated columns

required locally and/or transiently for insulin release but the measurement using quin2 is unable to detect such a change in cytosolic $[\text{Ca}^{2+}]$.

The mechanism by which somatostatin inhibits hormone release has been extensively studied, especially in the endocrine cells of pituitary and pancreas. Earlier studies showed that the inhibition of glucose-induced insulin release by somatostatin was reversed by increasing Ca^{2+} concentration in the medium (6, 14), and that somatostatin inhibited the release of insulin (30) or of growth hormone induced by high K (9), suggesting that somatostatin reduced Ca^{2+} influx. This view was supported by the observations that somatostatin inhibited Ca^{2+} uptake induced by glucose in pancreatic islets (15, 30) and Ca^{2+} influx into several clonal cells strains (12). However, other investigators failed to observe any clear effects of somatostatin on Ca^{2+} fluxes in the pituitary (25) or pancreas (28). Using fluorescent indicators, somatostatin was reported to lower cytosolic $[\text{Ca}^{2+}]$ in pituitary cells (20) and in pancreatic islets (13, 21). Since the effect on cytosolic $[\text{Ca}^{2+}]$ was only transient (13), the other

mechanism may exist.

The adenylate cyclase system was also proposed to be involved in the inhibitory effect of somatostatin on hormone release. Several reports have suggested that somatostatin inhibits insulin release by lowering intracellular level of cAMP (30). This may be due to the inhibition by somatostatin of adenylate cyclase, as shown in islet homogenates (2). However this effect of somatostatin cannot explain its inhibitory action on insulin release enhanced by $(\text{Bu})_2\text{cAMP}$, a stable analog of cAMP (24). Thus, it is possible that somatostatin may also act at the sites subsequent to cAMP accumulation. In pituitary cells, it was suggested that somatostatin could exert its inhibitory effects on GH secretion independently of cyclic nucleotides metabolism (25). The view seems to be consistent with the finding that somatostatin inhibits cAMP-dependent protein kinase activity in islets and pituitary (10), or that somatostatin activates phosphoprotein phosphatases in the digestive tract (17).

In the present study, somatostatin did not inhibit Ca^{2+} -induced insulin release. This can not be

ascribed to the damage of somatostatin receptor by the collagenase and digitonin treatments because somatostatin does inhibit insulin release augmented by forskolin, cAMP or TPA. Thus the effects of somatostatin vary with the kind of second messenger systems by which insulin release is mediated. It was reported that inhibition of glucose-induced insulin release, cAMP accumulation and ^{45}Ca uptake by somatostatin was prevented by treating intact islets with pertussis toxin and that G-protein might be involved in the actions of somatostatin (8). However the effects of somatostatin on receptor-mediated events cannot explain its inhibitory action on insulin release augmented by cAMP or TPA. It has recently been reported that somatostatin inhibits Ca^{2+} -induced insulin release in permeabilized HIT-T15 cells (26) or Ca^{2+} -induced ACTH release in AtT-20 cells (11) when GTP is added to the medium. Thus the inability of somatostatin to inhibit Ca^{2+} -induced release under the present condition may be due to leakage of intracellular GTP. Taken together somatostatin is likely to affect insulin release in at least three steps in stimulus-secretion coupling; 1) precedent to an increase in cytosolic $[\text{Ca}^{2+}]$ or cAMP concentration in a G-protein-dependent manner, 2) subsequent to an increase in cytosolic $[\text{Ca}^{2+}]$ in a G-protein-dependent manner, and 3) subsequent to an increase in cytosolic cAMP concentration or activation of protein kinase C in a G-protein-independent manner.

It is of interest to note that somatostatin inhibits insulin release potentiated by $10\text{ }\mu\text{M}$ but not that by 5 mM cAMP (Fig. 4). The intracellular concentration of cAMP was reported to be $1\text{ }\mu\text{M}$ in mouse islet (5) and approximately $10\text{ }\mu\text{M}$ in rat islet (calculated by assuming an intracellular water space of 2 to 3 nl per islet) (4). Thus somatostatin can inhibit insulin release increased by a physiological concentration of cAMP. But 5 mM cAMP, which is currently used in intact islets, was too high for somatostatin to exert its effects in permeabilized islets.

Excellent technical assistance by Ms F. Furuta, Aichigakuin University, and excellent secretarial assistance by Ms T. Satake, Nagoya University, are gratefully acknowledged. These studies were supported in part by Research Grant from the Ministry of Education, Science and Culture, Japan.

Received 30 July 1993; and accepted 10 August 1993

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