SOMATOSTATIN AND INSULIN RELEASE FROM ISOLATED RAT PANCREATIC ISLETS IN RESPONSE TO D-GLUCOSE,L-LEUCINE,α-KETOISOCAPROIC ACID OR D-GLYCERALDEHYDE:EVIDENCE FOR A REGULATORY ROLE OF ADENOSINE-3',5'-CYCLIC MONOPHOSPHATE

Peter Schauder,Christopher McIntosh,Jann Arends,Rudolf Arnold,Heiko Frerichs and Werner Creutzfeldt

Department of Medicine,Division of Gastroenterology and Metabolism, University of Göttingen,Federal Republic of Germany

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SUMMARY

Somatostatin and insulin release from isolated rat pancreatic islets was stimulated by glucose,leucine or α-ketoisocaproic acid. D-glyceraldehyde stimulated insulin release but diminished the secretion of somatostatin.Glucagon and theophylline amplified the glucose-induced somatostatin release.

A regulatory role of the D-cell's adenylate cyclase/phosphodiesterase system for the release of somatostatin is suggested.Furthermore,stimulation as well as inhibition of somatostatin release might be of significance for the secretory function of the B-cell.

Glucose stimulates the release of somatostatin from isolated rat pancreatic islets(1).In this study the effect of leucine, α-ketoisocaproic acid and D-glyceraldehyde on the secretion of somatostatin was investigated and compared with the insulin releasing action of these compounds to determine possible interrelationships.
In addition,the effect of glucagon and theophylline on glucose-induced somatostatin release was investigated,since the adenylate cyclase/phosphodiesterase system may be involved in this release process.

MATERIALS AND METHODS

Fed,male Wistar rats(200-270 g) were used throughout the study. Bovine serum albumin was purchsed from Behringwerke A.G. Marburg,FRG; 125-i-insulin(specific activity 150-200 mCi/mg) from Farbwerke Hoechst A.G.,Frankfurt,FRG;crystalline rat insulin from NOVO Industri A.S. Copenhagen,Denmark.Crystalline porcine glucagon(lot 258-D30-128-4) was a gift from Eli Lilly Co.,Indianapolis,USA.Cyclic somatostatin standard and 1-Tyr-Somatostatin were kindly provided by Dr.Romandini and Mr. Harrant;Serono,Freiburg,FRG.Collagenase was purchased from Worthington, Biochemical Co.,Freehold,New Jersey,USA.

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Table 1. Effect of D-glucose,L-leucine, α-ketoisocaproic acid or D-glyceraldehyde on somatostatin and insulin release from isolated rat pancreatic islets.Mean values ± SEM are shown with the number of individual observations obtained from 15-50 pancreata.An asterisk indicates a significant difference from the buffer control in line 1 ($P<0.02$ or less).Values for "P" were calculated by the "t" test based on nonpaired comparisons.

Glucose (mM)	Leucine (mM)	α-ketoisocaproic acid (mM)	D-glyceraldehyde (mM)	IR-Insulin (ng/10 islets/45 min)	IR-Somatostatin (pg/10 islets/45 min)
—	—	—	—	12.3 ± 0.6 (153)	24.5 ± 1.4 (153)
5.0	—	—	—	16.8 ± 1.1 (38)*	25.0 ± 3.0 (38)
8.0	—	—	—	35.9 ± 2.2 (91)*	29.4 ± 2.3 (91)
16.6	—	—	—	69.2 ± 2.8 (161)*	37.1 ± 2.0 (161)*
25.0	—	—	—	76.6 ± 3.2 (85)*	45.0 ± 4.5 (85)*
—	5.0	—	—	13.4 ± 1 0 (54)	22.2 ± 2.5 (54)
—	25.0	—	—	32.3 ± 3 4 (59)*	32.2 ± 4.5 (59)*
—	—	5.0	—	24.5 ± 1.6 (102)*	27.1 ± 3.0 (102)
—	—	25 0	—	80.2 ± 3.7 (160)*	35.0 ± 2.8 (160)*
—	—	—	3.0	16.8 ± 1.0 (99)*	12.2 ± 1.5 (99)*
—	—	—	10.0	36.3 ± 2.2 (88)*	3.2 ± 0.7 (88)*

Islets were isolated from rat pancreas by collagenase 2 h after intraperitoneal administration of 0.6 ml pilocarpine hydrochloride (2% w/v),as previously described(2,3),and incubated in batches of 10 in 500 µl Krebs-Ringer bicarbonate buffer with D-glucose,L-leucine, α-ketoisocaproic acid,D-glyceraldehyde,glucagon or theophylline.The respective concentrations are indicated in the tables.

Insulin released into the medium was determined by radioimmunoassay with rat insulin as the reference standard.Insulin release is expressed as ng/10 islets/45 min.Somatostatin was measured by radioimmunoassay using synthetic cyclic somatostatin as standard.Antibodies were raised in rabbits by immunisation with synthetic cyclic somatostatin conjugated to bovine serum albumin with 1-ethyl-3(3-dimethylamino-propyl)carbodiimide HCL(4).1-Tyr-somatostatin was labelled with 125-I by the chloramin-T procedure(5) and separated from free 125-I and damaged hormone by adsorption to Quso G32 and elution with acetic acid/acetone/water(1:39:40).The labelled peptide was lyophilized and purified prior to each assay on CM-cellulose (Whatman CM 52) columns(6).Assays were performed in 50 mM barbital and human serum albumin(1%).Bound and free hormone were separated with dextran coated charcoal.The sensitivity of the assay is 200 fg per assay tube.No cross reaction is seen with the following hormones: Cholecystokinin,gastrin,glucagon,insulin,proinsulin,secretin,thyroid stimulating hormone or LH-releasing hormone.Somatostatin release is expressed as pg/10 islets/45 min.

RESULTS

The effect of glucose,leucine, α-ketoisocaproic acid and D-glyceraldehyde on the release of somatostatin and insulin is shown in table 1.The stimulatory effect of glucose is dose-dependant,rea-

Table 2. Effect of theophylline or glucagon on glucose-induced somatostatin and insulin release from isolated rat pancreatic islets. Mean values ± SEM are shown with the number of individual observations obtained from islets of 10-30 pancreata. An asterisk indicates a significant difference from the respective control, where theophylline or glucagon is omitted ($P < 0.01$ or less). Values for "P" were calculated by the "t" test based on nonpaired comparisons.

Glucose (mM)	Theophylline (mM)	Glucagon (ug/ml)	IR-Insulin (ng/10 islets/45 min)	IR-Somatostatin (pg/10 islets/45 min)
—	—	—	12.1 ± 1.1 (59)	23.6 ± 2.2 (59)
—	5	—	16.4 ± 1.0 (68)*	38.2 ± 3.6 (68)*
8.0	—	—	33.9 ± 2.4 (52)	30.3 ± 3.9 (52)
8.0	5	—	53.3 ± 4.3 (73)*	50.4 ± 3.8 (73)*
16.6	—	—	72.3 ± 4.3 (75)	37.2 ± 2.3 (75)
16.6	5	—	104.1 ± 4.8 (68)*	57.0 ± 5.0 (68)*
—	—	—	13.3 ± 1.2 (35)	24.0 ± 2.3 (35)
—	—	5	12.5 ± 1.2 (36)	23.3 ± 1.8 (36)
8.0	—	—	35.8 ± 3.6 (39)	28.1 ± 2.1 (39)
8.0	—	5	47.4 ± 4.9 (39)*	45.1 ± 5.0 (39)*
16.6	—	—	66.8 ± 3.7 (87)	37.3 ± 2.5 (87)
16.6	—	5	92.3 ± 6.2 (67)*	43.0 ± 2.3 (67)

ching a maximum at a concentration of 25 mM. At this concentration, insulin release is increased about 6-fold, somatostatin release about 2-fold. The threshold concentration of glucose for stimulation of insulin release is below 5 mM, for stimulation of somatostatin release above 8 mM. Leucine and α-ketoisocaproic acid both enhance the secretion of somatostatin. By contrast, D-glyceraldehyde (3 or 10 mM) diminished the release of somatostatin. At these concentrations, insulin release is increased.

The effect of theophylline and glucagon on glucose-induced somatostatin release is shown in table 2. In a glucose-free buffer, theophylline stimulates insulin release only poorly, but has a pronounced stimulatory action on the release of somatostatin. Theophylline amplifies the stimulatory effect of 8 and 16.6 mM glucose on the release of somatostatin. Glucagon influences neither the secretion of insulin nor the release of somatostatin in the absence of glucose, but enhances the effect of 8.0 mM glucose on the secretion of both hormones. In the presence of 16.6 mM glucose, only insulin release is amplified by the addition of glucagon (table 2).

DISCUSSION

The present data indicate that glucagon and theophylline amplify the stimulatory effect of glucose on somatostatin release from iso-

lated rat pancreatic islets(table 2),thereby confirming previous results(7).This suggests a regulatory role of adenosine-3',5'-cyclic monophosphate for the release process of somatostatin,assuming a stimulatory action of glucagon on adenylate cyclase activity and an inhibitory effect of theophylline on phosphodiesterase activity in D-cells.This is supported by the observation that 8-Br-cyclic AMP stimulates somatostatin release in the presence of 100 or 300 mg/100 ml glucose(8).The concentration of theophylline(5 mM) and glucagon (5 μg/ml) employed is high.Under these conditions,theophylline has a greater effect on somatostatin release than glucagon.Moreover, unlike glucagon,theophylline is effective in the absence of glucose. If the effect of theophylline and glucagon on the adenylate cyclase/ phosphodiesterase system is maximal,the data might indicate that somatostatin release is increased more via inhibition of the D-cell's phosphodiesterase system than via stimulation of the adenylate cyclase activity.

Stimulation of somatostatin release by glucose is about 2-fold and dose-dependant(table 1).This effect of glucose was not observed by others(8).The reason for this is not apparent,but might be due to the different procedures of isolation and incubation of islets. Glucose is not the only stimulator of somatostatin release.Leucine and α-ketoisocaproic acid are effective as well,although less than glucose.Whether these compounds enhance the release of somatostatin by direct stimulation of the D-cells,or whether their stimulatory effect is mediated by insulin remains undecided.

D-glyceraldehyde at low concentrations stimulates the release of insulin,but is inhibitory at high concentrations(9-14).However,while is enhanced in the presence of low D-glyceraldehyde concentrations (3 or 10 mM),somatostatin release is diminished(table 1).These observations might indicate a different metabolic regulation of the B- and D-cells.Whether this merely reflects a higher sensitivity of D-cells compared with B-cells to the ATP-lowering action of D-glyceraldehyde in islets(9),or whether a more specific mechanism is operating,remains to be investigated.

We previously suggested that the high "basal release" by somatostatin containing cells might be due to leakage,explained by the location of D-cells in the periphery of the islets where damage during isolation is greatest(1).However,the present data indicate that "basal release" of somatostatin,unlike "basal release" of insulin,can be inhibited almost completely(table 1).Therefore,the comparatively high

somatostatin levels in the medium seen under "basal conditions",i.e. in the absence of glucose,might reflect active release rather than leakage.It has been suggested that insulin release stimulates somatostatin release(1).If this is the case,the high rate of somatostatin secretion in the absence of glucose might be due to a stimulatory action of insulin released under "basal conditions",i.e. in the absence of glucose.The "basal release" of somatostatin observed would actually be already insulin-stimulated release.
According to the data presented here the D-cell function can significantly increase or decrease,thus,stimulation as well as inhibition of somatostatin release might be of importance for the secretory function of the B-cell.

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