Intra-Islet Somatostatin Regulates Glucagon Release via Type 2 Somatostatin Receptors in Rats

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Exogenously administered somatostatin (SST) inhibits secretion of insulin and glucagon. Furthermore, it is hypothesized that islet SST regulates glucagon secretion by a local action. A number of studies utilizing SST antibodies have been performed to test this hypothesis, and their results have been conflicting. Five subtypes of SST receptor (SSTR1–5) mediate the effect of SST on target cells. In rodents, SST inhibits the release of glucagon, but not that of insulin, via SSTR2. A novel SSTR2-selective antagonist, DC-41-33, was synthesized recently. We have investigated the effects of this antagonist on arginine-stimulated glucagon and insulin release in batch incubations of isolated rat islets, perifused isolated rat islets, and isolated perfused rat pancreas. In batch incubations at 3.3 mmol/l glucose, DC-41-33 increased glucagon release in a dose-dependent manner. At the maximum dose tested (2 μmol/l), DC-41-33 enhanced the glucagon response by 4.3- to 5-fold. Similarly, this compound increased arginine-induced glucagon release in perifused islets at 3.3 mmol/l glucose (2.8-fold) and perfused pancreas at 3.3 and 5.5 mmol/l glucose (2.5- and 2.3-fold, respectively). In the two latter experimental systems, DC-41-33 had no significant effect on insulin release. In conclusion, our results strongly support the hypothesis that islet SST inhibits glucagon secretion via a local action. *Diabetes* 52:1176–1181, 2003

It is well established that exogenously administered somatostatin (SST) inhibits the release of insulin (1,2) and glucagon (3,4) from the endocrine pancreas. In addition, it has been hypothesized that SST released from islet D-cells regulates the secretion of insulin and glucagon by a local "paracrine" action (5,6). This hypothesis was supported by experiments demonstrating that immunoneutralization of endogenous SST in batch incubations of isolated pancreatic islets enhances glucagon and insulin secretion (7,8). However, the results of these studies do not necessarily depict physiological events, since released hormones accumulate in the medium and islet interstitium during the 30- to 90-min incubation period, reaching concentrations that most probably exceed those that are physiologically relevant. Additionally, isolated islets retain neither their normal interstitial structure nor their microcirculation. Further support for the involvement of local effects of SST came from an in vivo study in dogs, in which low and medium doses of a nonimmunoreactive SST analog suppressed the release of endogenous SST and markedly enhanced glucagon release, while insulin release was enhanced moderately (9). The possibility remains, however, that this analog had systemic effects that influenced hormone release.

For the reasons stated above, studies with isolated perfused pancreas are of special relevance when considering paracrine interactions between islet cells. In the perfused isolated canine pancreas, exogenous SST inhibited arginine-stimulated insulin and glucagon secretion at concentrations as low as 20% of those measured in venous effluent (10). The authors interpreted this finding as indicating that islet SST receptors (SSTRs) are not in contact with high local concentrations of endogenous SST. The integrity of this separation determines the sensitivity of islet cells to circulating SST. The study thus argued against a local regulatory role for SST in the islet (11). This conclusion is supported by a series of immunoneutralization studies in isolated perfused pancreata of rat, dog, and humans, in which anterograde perfusion with polyclonal SST antibodies did not significantly affect either insulin or glucagon secretion (12–16). In contrast, Brunicardi and colleagues (17,18) reported that neutralization of intra-islet SST with monoclonal antibodies enhances both glucagon and insulin secretion from isolated perfused human pancreas.

Five subtypes of SSTRs (SSTR1–5) mediate the effect of SST on target cells (19,20). Previous studies in rats have indicated that SST inhibits glucagon and insulin release via SSTR2 and SSTR5, respectively (21–24). The recent development of a specific SSTR2 antagonist, DC-41-33, also known as PRL-2903 (25), gave us the unique possibility to explore whether islet SST regulates glucagon release locally. For this purpose, we first characterized the effects of DC-41-33 on glucagon and insulin release in batch incubations of isolated rat islets and then applied DC-41-33 to perifused isolated rat islets and isolated perfused rat pancreata.

RESEARCH DESIGN AND METHODS

Animals. Male Wistar rats, aged 2–3 months, were obtained from B&K Universal (Sollentuna, Sweden). The animals were fed ad libitum with free access to water and placed in rooms with alternate 12-h periods of light and darkness. The experiments were approved by the Ethical Committee of the Karolinska Institute (ethical application no. N80/01). The guidelines of the

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KRBB, Krebs-Ringer bicarbonate buffer; SST, somatostatin; SSTR, SST receptor.

Swedish Research Council for the use and care of laboratory animals were followed.

Isolation of pancreatic islets, batch incubation, and perifusion studies. Islets were isolated by digestion with collagenase (Hoffmann-La Roche, Basel, Switzerland) and cultured for 20–22 h in RPMI-1640 medium supplemented with 11 mmol/l glucose and 10% (vol/vol) FCS. For both batch incubation and perifusion studies, the islets were first preincubated for 35 min in Krebs-Ringer bicarbonate buffer (KRBB) containing 3.3 mmol/l glucose and 2 g/l bovine plasma albumin (Sigma, St. Louis, MO). Batches of ten islets were then incubated for 1 h in 350 μl KRBB-albumin-glucose. As a stimulus for hormone release, 20 mmol/l arginine was used. DC-41-33 and/or SST (SST-14; Sigma, St. Louis, MO) were added to incubations with arginine. After each incubation, 100-μl aliquots of incubation medium were stored at −20°C for subsequent radioimmunoassay of insulin (26) and glucagon (27).

After culture and preincubation, 100 islets were added to each of two perifusion chambers by layering between inert polystyrene beads (Bio-Gel 200-400 mesh; Bio-Rad Laboratories, Richmond, CA). This perifusion system has been previously described (28). As a basal perifusion medium, we used the KRBB-albumin-glucose as described above, with the flow rate of 0.4 ml/2 min. The perifusion protocol was started by a 30-min equilibration period with basal medium, followed by a 20-min stimulation period, and finally by a 10-min reperifusion with the basal medium. Arginine at a concentration of 20 mmol/l was used as the stimulus for hormone release. Samples were collected at 2-min intervals, ice-chilled immediately, frozen, and kept at −20°C until analysis (described above).

Perfusion of isolated rat pancreas. Each animal was anesthetized with an intraperitoneal injection of sodium thiopental (100 mg/kg body wt). The pancreata were dissected away from adjacent tissues, as described previously (29). An open, nonrecycling perfusion system was used to administer perfusion medium via a cannula inserted in the abdominal aorta. The perfused pancreas was mounted on the cannula within the perfusion chamber. The temperature in the chamber was maintained at 37°C. Samples of the perfusate emerging from the portal vein were collected at 1-min intervals. The basal perfusion medium consisted of KRBB (in mmol/l: 115 NaCl, 4.7 KCl, 2.6 $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, and 20 $NaHCO_3$) supplemented with 3.3 or 5.5 mmol/l glucose and 20 g/l BSA. In one series of experiments, the buffer was supplemented with only 20% of the standard calcium content (Fig. 6). The medium was continuously gassed with a mixture of 95% O_2:5% CO_2. Pancreata were first perfused with this basal medium for a 20-min equilibration period. During the following 10 min, either 1 μmol/l DC-41-33 or 10 nmol/l SST-14 (Sigma) were added to the perfusion medium, except for control experiments. Arginine (20 mmol/l) was then added for a 20-min stimulation period. Finally, basal medium only was perfused for the last 10 min of the protocol. The flow rate was maintained at 3 ml/min, and variations from 2.8 to 3.2 ml/min were only occasionally observed. Results were calculated as the concentration of hormone per milliliter of perfusate multiplied by the flow rate. Samples were collected in ice-chilled tubes containing 1,000 units aprotinin (Trasylol; Bayer, Leverkusen, Germany) at 1-min intervals, frozen, and kept at −20°C for analysis as above.

Statistics. Results are expressed as means ± SE. One- and two-way ANOVA, followed by Dunnett's test and Newman-Keul's test, respectively, were used to evaluate treatment effects in experiments with batch-incubated islets. The results of perifusion and perfusion studies were calculated as the area under the curve, and statistical analyses were assessed by unpaired t test. A $P < 0.05$ was considered to be significant. Statistical analyses were performed using the programs Statistica (StatSoft, Tulsa, OK) and SigmaPlot 2000 for Windows, version 6.0 (SPSS).

RESULTS

Hormone release from isolated islets in batch incubations. In batch incubations of isolated islets at 3.3 mmol/l glucose, DC-41-33 was added at concentrations of 10–2,000 nmol/l (Fig. 1). This antagonist had a slight but significant stimulatory effect on the insulin response to 20 mmol/l arginine only at concentrations of 100 nmol/l (0.19 ± 0.01 vs. 0.23 ± 0.01 pmol · islet^{-1} · h^{-1}, $P < 0.05$). At a concentration of ≥100 nmol/l, DC-41-33 markedly stimulated the glucagon response to arginine (26.1 ± 2.9 vs. 76.5 ± 5.8 pg · islet^{-1} · h^{-1}, $P < 0.001$), and this increase was dose dependent. The two highest doses tested, 1 and 2 μmol/l DC-41-33, enhanced glucagon release by 3.8- and 4.3-fold, respectively.

SST (10–1,000 nmol/l) had no significant effect on the insulin response in batch-incubated islets (Fig. 2 and Table 1) at 20 mmol/l arginine. However, the addition of 2 μmol/l DC-41-33 enhanced the arginine-stimulated insulin response ($P < 0.001$) in the absence or presence of exogenous SST in the medium. In these experiments, inhibition

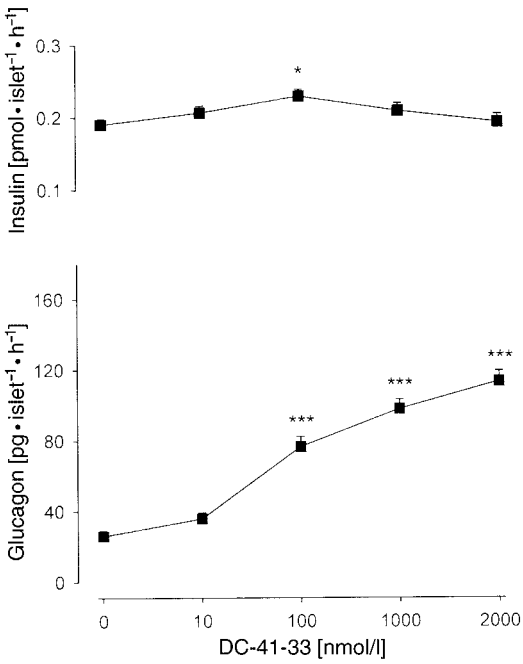

FIG. 1. The effect of DC-41-33 on insulin and glucagon response to 20 mmol/l arginine in batch incubations of isolated islets at 3.3 mmol/l glucose. Results are expressed as mean ± SE of three experiments (three to five observations per experiment). *$P < 0.05$, ***$P < 0.001$ vs. 0 nmol/l DC-41-33.

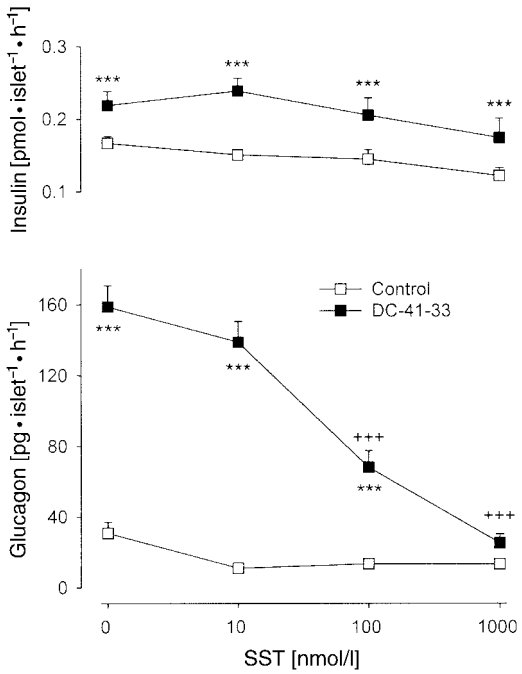

FIG. 2. The effect of 2 μmol/l DC-41-33 on the insulin and glucagon responses to 20 mmol/l arginine in the presence of increasing concentrations of SST, in batch incubations of isolated islets. Results are expressed as mean ± SE of three experiments (three to five observations per experiment). ***$P < 0.001$ vs. without DC-41-33; $^{+++}P < 0.001$ vs. 0 nmol/l SST + 2 μmol/l DC-41-33.

TABLE 1
The effect of DC-41-33 on hormonal responses in the presence of SST, in batch-incubated islets

SST (nmol/l)	DC-41-33 (μmol/l)	Insulin (pmol · islet^{-1} · h^{-1})	Glucagon (pg · islet^{-1} · h^{-1})
0	0	0.17 ± 0.01	30.8 ± 6.2
0	2	0.22 ± 0.02*	158.7 ± 11.9*
10	0	0.15 ± 0.01	11.0 ± 1.1
10	2	0.24 ± 0.02*	138.7 ± 11.7*
100	0	0.14 ± 0.01	13.4 ± 2.5
100	2	0.20 ± 0.02*	68.1 ± 9.4*†
1,000	0	0.12 ± 0.01	13.3 ± 1.9
1,000	2	0.17 ± 0.03*	25.2 ± 5.1†

Data were obtained from the batch incubations shown in Fig. 2 and are presented as means ± SE of three experiments (three to five observations per experiment). *$P < 0.001$ vs. without DC-41-33; †$P < 0.001$ vs. 0 nmol/l SST + DC-41-33.

of the glucagon response by SST (to 36, 44, and 43% of controls at 10, 100, and 1,000 nmol/l SST, respectively) failed to reach significance. At a concentration of 2 μmol/l, DC-41-33 enhanced arginine-induced glucagon release by fivefold. In the presence of DC-41-33, 10 nmol/l SST had no significant effect on glucagon release, but at concentrations of 100 nmol/l and 1 μmol/l, SST inhibited the release of glucagon by 57 and 84%, respectively ($P < 0.001$).

Hormone release from isolated perifused islets and perfused pancreas. Arginine (20 mmol/l) induced significant insulin and glucagon release from isolated perifused rat islets at 3.3 mmol/l glucose. DC-41-33 had no effect on the insulin response to arginine, but enhanced arginine-induced glucagon release by 2.8-fold (area under the curve 3,219.5 ± 403.5 vs. 8,966.9 ± 1,485.7 pg, respectively, $P < 0.01$).

In the isolated perfused pancreas, 20 mmol/l arginine induced biphasic insulin and glucagon responses at 3.3 and 5.5 mmol/l glucose (Figs. 4 and 5, respectively). DC-41-33 (1 μmol/l) had no effect on insulin release at 3.3 mmol/l glucose (Fig. 4), but it enhanced the second phase of insulin response at 5.5 mmol/l glucose by 34.6% (Fig. 5). This effect was not significant ($P = 0.4$). The antagonist stimulated glucagon release by 2.5-fold at 3.3 mmol/l glucose and 2.3-fold at 5.5 mmol/l glucose (Table 2). The first phase of glucagon response was not significantly affected by DC-41-33 at either of these two glucose concentrations, while the second phase (4–20 min) was stimulated by 2.7-fold ($P < 0.02$) and 2.4-fold ($P < 0.005$) at 3.3 and 5.5 mmol/l glucose, respectively. However, the peak glucagon response at 3.3 mmol/l glucose, achieved at the second minute of arginine stimulation, was enhanced twofold by DC-41-33 (4,735.4 ± 826.8 vs. 9,750.2 ± 1,018.5 pg/min, controls vs. DC-41-33, respectively, $P < 0.01$) (Fig. 4).

To abolish the release of endogenous SST, we perfused pancreata with buffer supplemented with only 20% of the standard calcium content (30). In these experiments, at 3.3 mmol/l glucose, insulin and glucagon each responded to arginine in a biphasic manner, while DC-41-33 did not significantly affect these hormonal responses (Fig. 6). Addition of 10 nmol/l SST-14 to the perfusate completely abolished the release of insulin and glucagon, both in basal conditions and in the presence of arginine.

DISCUSSION

SST is produced and secreted in various organ systems, such as the endocrine pancreas, gastrointestinal tract, and the brain, where it regulates endocrine and exocrine secretion, cell division, and neurotransmission (31). Of the two endogenous bioactive forms of SST (SST-14 and -28), SST-14 is the predominant form in pancreatic islets (31). Both forms of SST inhibit the secretion of glucagon and insulin, but SST-14 inhibits glucagon release more potently than that of insulin (16). SST exerts its effect on target cells via G-protein–coupled receptors, which are encoded for by five genes. Each of these genes expresses a single protein, except for SSTR2, which has two splice variants, SSTR2a and -b. These differ only in length and amino-acid sequence at the carboxy terminus (19,20,32). An immunohistochemical study of human islets identified SSTR2 as the predominant subtype expressed in A-cells, and SSTR1 and SSTR5 as the predominant subtypes in B-cells (33). Similarly, such studies in rats localized SSTR2 to the A-cells and SSTR5 to the B-cells (21,22). Also, pharmacological studies in rats utilizing subtype-specific SSTR agonists demonstrated that SSTR2 mediates the action of SST on A-cells, while SSTR5 carries out this role in B-cells (23,24).

DC-41-33 is a highly specific SSTR2 antagonist (25). In CHO cells transfected with human SSTRs, this compound binds competitively to SSTR2 with a binding affinity (K_i) of 26 ± 3.1 nmol/l and displays selectivity for human SSTR2 over SSTR5 by factor 20 (25). In primary cultures of rat pituitary cells, growth hormone release factor–stimulated

TABLE 2
The effect of DC-41-33 on hormonal release in perfused pancreas

Min	DC-41-33 (μmol/l)	3.3 mmol/l glucose		5.5 mmol/l glucose		20% of the standard Ca^{2+} 3.3 mmol/l glucose	
		Insulin	Glucagon	Insulin	Glucagon	Insulin	Glucagon
0–4	0	14.4 ± 2.9	9.6 ± 2.2	30.6 ± 5.0	4.7 ± 1.2	8.1 ± 1.5	8.2 ± 1.6
0–4	1	9.8 ± 1.3	16.9 ± 2.2	24.7 ± 8.3	7.0 ± 1.2	3.7 ± 1.4	6.3 ± 1.4
4–20	0	38.2 ± 6.2	33.3 ± 7.5	89.0 ± 14.6	19.1 ± 5.5	12.7 ± 2.3	28.1 ± 8.2
4–20	1	38.2 ± 7.2	91.1 ± 20.2*	119.8 ± 36.3	47.0 ± 6.1†	14.1 ± 3.6	32.8 ± 7.4
0–20	0	52.6 ± 8.7	43.0 ± 9.6	119.7 ± 18.8	23.4 ± 6.4	20.8 ± 3.3	36.3 ± 9.5
0–20	1	47.9 ± 8.4	108.0 ± 22.2*	144.5 ± 44.4	54.1 ± 7.2‡	17.7 ± 4.7	39.1 ± 8.8

Data were obtained from the perfusion experiments shown in Figs. 4–6 and represent the amounts of insulin (pmol) and glucagon (ng) released during the 20-min stimulation period. Arginine (20 mmol/l) was used as the stimulus. The hormone amount is calculated as the area under the curve, using the secretion rate at min 0 as the basal value. *$P < 0.02$, †$P < 0.005$, and ‡$P < 0.01$ vs. without DC-41-33.

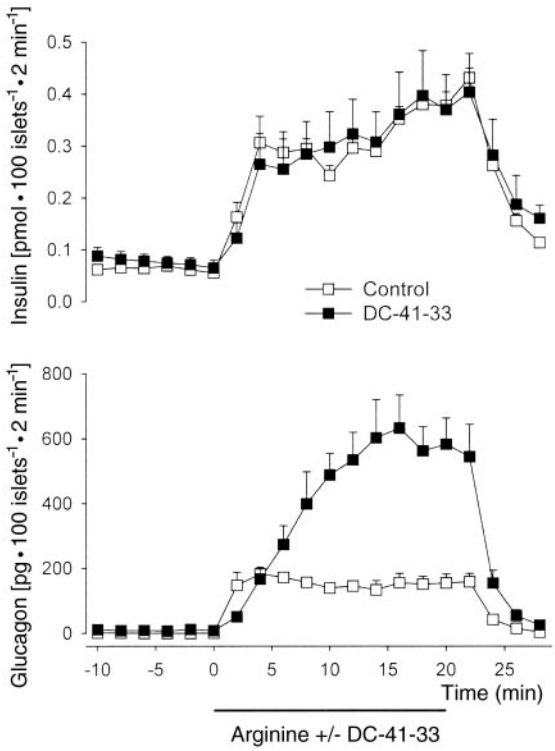

FIG. 3. The effect of 2 μmol/l DC-41-33 on insulin and glucagon release induced by 20 mmol/l arginine in perifused isolated islets. Results are expressed as mean ± SE of four experiments.

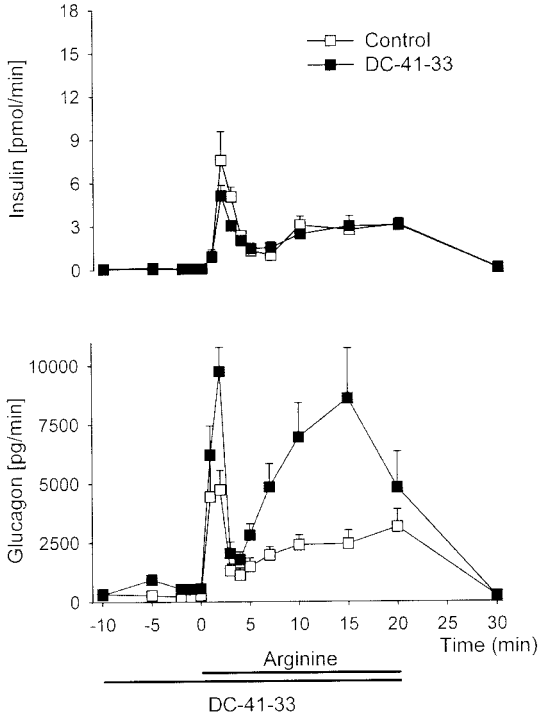

FIG. 4. The effect of DC-41-33 (1 μmol/l) on insulin and glucagon release induced by 20 mmol/l arginine in isolated perfused pancreas at 3.3 mmol/l glucose. Results are expressed as mean ± SE. Controls: n = 6; DC-41-33: n = 4.

growth hormone release was inhibited by 1 nmol/l SST. This inhibition was potently reversed by DC-41-33, with a half-maximal inhibitory concentration of 2.5 nmol/l (25). The use of DC-41-33 also allows a dissection of the acute effects of SST in events mediated by SSTR2, such as the inhibition of gastric acid secretion and certain hormonal responses. Hence, in vivo studies have demonstrated that DC-41-33 blocks intravenous SST-induced inhibition of pentagastrin-stimulated acid secretion in conscious rats (34) and reverses urethane-induced SST-mediated inhibition of gastric acid secretion (35).

In the present study, application of DC-41-33 in batch incubations of isolated rat islets induced a dose-dependent enhancement of arginine-stimulated glucagon release (Fig. 1). Furthermore, 2 μmol/l DC-41-33 antagonized the inhibitory action of 10 nmol/l SST on arginine-induced glucagon release. Since this concentration of SST is at least 5- to 50-fold higher then that estimated to be present in islet capillaries and interstitium (10,16), we assumed that 2 μmol/l DC-41-33 should abolish the effect of islet SST on glucagon release in our perifusion experiments (Fig. 3). The lower but nearly equipotent dose, 1 μmol/l, was used in perfused pancreas, because delivery of DC-41-33 was expected to be more efficient by islet microcirculation, which is preserved in this model (Figs. 4–6). Under these experimental conditions, DC-41-33 markedly increased the glucagon response to arginine in both isolated perifused islets and perfused rat pancreas. Since we have used a nonrecycling perifusion and perfusion system, these findings suggest that islet SST regulates glucagon release via a local action in rats. This may apply equally at both fasted and nonfasted conditions, since DC-41-33 enhanced the glucagon response of perfused pancreata to a similar degree at 3.3 and 5.5 mmol/l glucose (2.5- and 2.3-fold, respectively). We do not know whether the octapetide DC-41-33 is able to penetrate the capillary wall and pass into the islet interstitium in these experiments. Therefore,

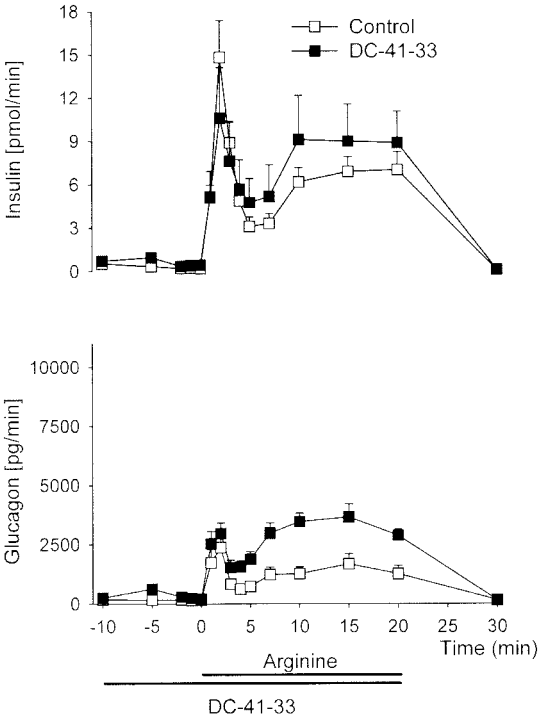

FIG. 5. The effect of DC-41-33 (1 μmol/l) on insulin and glucagon release induced by 20 mmol/l arginine in isolated perfused pancreas at 5.5 mmol/l glucose. Results are expressed as mean ± SE. Controls: n = 10; DC-41-33: n = 7.

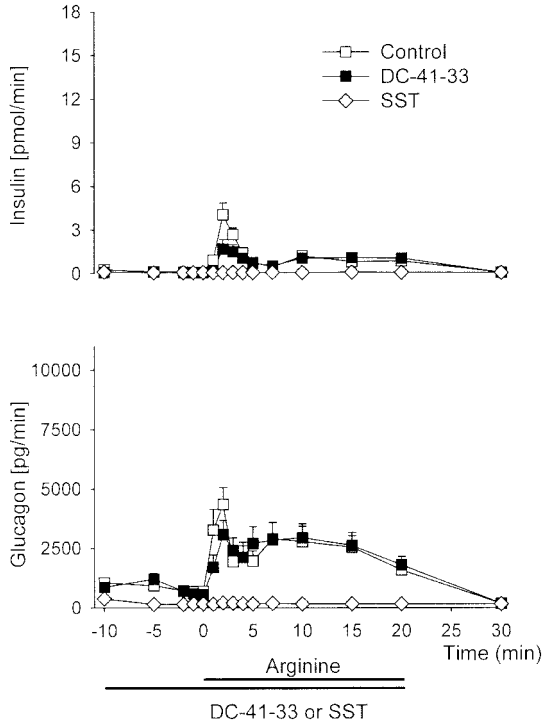

FIG. 6. The effect of DC-41-33 (1 μmol/l) or SST (10 nmol/l) on insulin and glucagon release induced by 20 mmol/l arginine in isolated perfused pancreas at 3.3 mmol/l glucose and 20% of the standard calcium content in the perfusion medium. Results are expressed as mean ± SE. Controls: $n = 9$; DC-41-33: $n = 8$; SST: $n = 6$.

it is not clear whether SST secreted from D-cells exerts its local effect on A-cells directly via the islet interstitum by paracrine action and/or via the islet microcirculatory system by a short-loop endocrine interaction. The hypothesis that islet SST regulates glucagon release via a local action is supported by the present findings that DC-41-33 failed to stimulate arginine-induced glucagon release in experiments with perfusion medium containing only 20% of the standard calcium content (Fig. 6). This procedure is known to blunt the endogenous SST response (30). Our findings are consistent with the results of a study using batch-incubated islets isolated from SSTR2-knockout mice, in which the glucagon response to arginine and potassium was enhanced twofold, while insulin release was unchanged (36). Furthermore, in a recent in vivo study, administration of another SSTR2-specific antagonist, BIM-23627, increased plasma glucagon levels in 10-day-old, freely moving rats (37).

In the experiments with perifused islets or isolated perfused pancreata at 3.3 mmol/l glucose, blockade of SSTR2 had no effect on insulin release. However, in perfused pancreata at 5.5 mmol/l glucose, DC-41-33 increased the second phase of insulin release by 34.6% ($P = 0.4$). Although this compound shows 20-fold higher affinity to SSTR2 (A-cells) than SSTR5 (B-cells) (25), it is possible that the antagonist enhanced glucagon as well as insulin release in experiments with higher basal glucose in the perfusate that facilitates SST release. Significant enhancements of insulin secretion occurred in most but not all of our batch incubations of isolated islets at 2 μmol/l DC-41-33. Currently, we have no obvious explanation for this phenomenon. This finding may be accounted for by a synergistic insulinotropic effect of arginine and glucagon, accumulating in the medium during the 1-h incubation time.

In conclusion, our study demonstrates for the first time the local inhibitory effect of islet SST on arginine-stimulated glucagon release in intact rat islets in vitro. This inhibition is mediated by SSTR2. Further studies are needed to explore the physiological significance and mechanisms of this interaction in rats, as well as in other species.

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REFERENCES

1. Alberti KG, Christensen NJ, Christensen SE, Hansen AP, Iversen J, Lundbaek K, Seyer-Hansen K, Orskov H: Inhibition of insulin secretion by somatostatin. *Lancet* 2:1299–1301, 1973
2. Efendic S, Luft R, Grill V: Effect of somatostatin on glucose induced insulin release in isolated perfused rat pancreas and isolated rat pancreatic islets. *FEBS Lett* 42:169–172, 1974
3. Gerich JE, Lorenzi M, Schneider V, Kwan CW, Karam JH, Guillemin R, Forsham PH: Inhibition of pancreatic glucagon responses to arginine by somatostatin in normal man and in insulin-dependent diabetics. *Diabetes* 23:876–880, 1974
4. Koerker DJ, Ruch W, Chideckel E, Palmer J, Goodner CJ, Ensinck J, Gale CC: Somatostatin: hypothalamic inhibitor of the endocrine pancreas. *Science* 184:482–484, 1974
5. Unger RH, Orci L: Possible roles of the pancreatic D-cell in the normal and diabetic states. *Diabetes* 26:241–244, 1977
6. Efendic S, Lins PE, Luft R, Uvnäs-Wallensten K, Szecowka J: Somatostatin: paracrine or endocrine substance? *Front Hormone Res* 7:41–51, 1980
7. Barden N, Lavoie M, Dupont A, Cote JP: Stimulation of glucagon release by addition of antisomatostatin serum to islets of Langerhans *in vitro*. *Endocrinology* 101:635–638, 1977
8. Itoh M, Mandarino L, Gerich JE: Antisomatostatin gama globulin augments secretion of both insulin and glucagon *in vitro*: evidence for a physiologic role for endogenous somatostatin in the regulation of pancreatic A- and B-cell function. *Diabetes* 29:693–696, 1980
9. Taborsky GJ Jr: Evidence for a paracrine role for pancreatic somatostatin in vivo. *Am J Physiol* 245:E598–E603, 1983
10. Kawai K, Ipp E, Orci L, Perrelet A, Unger RH: Circulating somatostatin acts on the islets of Langerhans by way of a somatostatin-poor compartment. *Science* 218:477–478, 1982
11. Unger RH, Orci L: Glucagon. In *Ellenberg & Rifkin's Diabetes Mellitus*. 5th ed. Porte D, Sherwin RS, Eds. Stamford, CT, Appleton & Lange, 1997, p. 115–139
12. Samols E, Stagner JI, Ewart RB, Marks V: The order of islet microvascular cellular perfusion is B–A–D in the perfused rat pancreas. *J Clin Invest* 82:350–353, 2001
13. Stagner JI, Samols E, Bonner-Weir S: β → α → δ pancreatic islet cellular perfusion in dogs. *Diabetes* 37:1715–1721, 1988
14. Stagner JI, Samols E: The vascular order of islet cellular perfusion in the human pancreas. *Diabetes* 41:93–97, 1992
15. Samols E, Stagner JI: Intra-islet cell-cell interactions and insulin secretion. *Diabetes Reviews* 4:207–223, 1996
16. Samols E, Stagner JI: Intraislet and islet acinar portal systems and their significance. In *The Endocrine Pancreas*. Samols E, Ed. New York, Raven Press, 1991, p. 93–124
17. Brunicardi FC, Kleinman R, Moldovan S, Nguyen TH, Watt PC, Walsh J,

Gingerich R: Immunoneutralization of somatostatin, insulin, and glucagon causes alterations in islet cell secretion in the isolated perfused human pancreas. *Pancreas* 23:302–308, 2001
18. Kleinman R, Gingerich R, Ohning G, Wong H, Olthoff K, Walsh J, Brunicardi FC: The influence of somatostatin on glucagon and pancreatic polypeptide secretion in the isolated perfused human pancreas. *Int J Pancreatol* 18:51–57, 1995
19. Patel YC, Srikant CB: Somatostatin receptors. *Trends Endocrinol Metab* 8:398–405, 1997
20. Csaba Z, Dournaud P: Cellular biology of somatostatin receptors. *Neuropeptides* 35:1–23, 2001
21. Hunyady B, Hipkin RW, Schonbrunn A, Mezey E: Immunohistochemical localization of somatostatin receptor SST2A in the rat pancreas. *Endocrinology* 138:2632–2635, 1997
22. Mitra SW, Mezey E, Hunyady B, Chamberlain L, Hayes E, Foor F, Wang Y, Schonbrunn A, Schaeffer JM: Colocalization of somatostatin receptor sst5 and insulin in rat pancreatic β-cells. *Endocrinology* 140:3790–3796, 1999
23. Rossowski WJ, Coy DH: Potent inhibitory effects of a type four receptor-selective somatostatin analog on rat insulin release. *Biochem Biophys Res Commun* 197:366–371, 1993
24. Rossowski WJ, Coy DH: Specific inhibition of rat pancreatic insulin or glucagon release by receptor-selective somatostatin analogs. *Biochem Biophys Res Commun* 205:341–346, 1994
25. Hocart SJ, Jain R, Murphy WA, Taylor JE, Coy DH: Highly potent cyclic disulfide antagonists of somatostatin. *J Med Chem* 42:1863–1871, 1999
26. Herbert V, Lau KS, Gottlieb CW, Bleicher SJ: Coated charcoal immunoassay of insulin. *J Clin Endocrinol Metab* 25:1375–1384, 1965
27. Faloona GR, Unger RH: Radioimmunoassay technique. In *Methods in Hormone Radioimmunoassay.* Vol 1. Jaffe BN, Behrmann HE, Eds. New York, Academic Press, 1974, p. 324–326
28. Kanatsuna T, Lernmark Å, Rubenstein AH, Steiner DH: Block in insulin release from calcium-perifused pancreatic beta-cells induced by islet cell surface antibodies and complement. *Diabetes* 30:231–234, 1981
29. Loubatieres AL, Mariani MM, Ribes G, De Malbosc H, Chapal J: Etude experimentale d'un nouveau sulfamide hypoglicemiant particulierement actif, le HB419 ou glibenclamide. *Diabetologia* 5:1–10, 1969
30. Östenson CG, Nylen A, Grill V, Gutniak M, Efendic S: Sulfonylurea-induced inhibition of glucagon secretion from the perfused rat pancreas: evidence for a direct, non-paracrine effect. *Diabetologia* 29:861–867, 1986
31. Reichlin S: Somatostatin. *N Engl J Med* 309:1495–1501, 1556–1563, 1983
32. Benali N, Ferjoux G, Puente E, Buscail L, Susini C: Somatostatin receptors. *Digestion* 62:27–32, 2000
33. Kumar U, Sasi R, Suresh S, Patel A, Thangaraju M, Metrakos P, Patel SC, Patel YC: Subtype-selective expression of the five somatostatin receptors (hSSTR1–5) in human pancreatic islet cells: a quantitative double-label immunohistochemical analysis. *Diabetes* 48:77–85, 1999
34. Rossowski WJ, Cheng BL, Jiang NY, Coy DH: Examination of somatostatin involvement in the inhibitory action of GIP, GLP-1, amylin and adrenomedulin on gastric acid release using a new SRIF antagonist analogue. *Br J Pharmacol* 125:1081–1087, 1998
35. Kawakubo K, Coy DH, Walsh JH, Tache Y: Urethane-induced somatostatin mediated inhibition of gastric acid: reversal by the somatostatin 2 receptor antagonist, PRL-2903. *Life Sci* 65:115–120, 1999
36. Strowski MZ, Parmar RM, Blake AD, Schaeffer JM: Somatostatin inhibits insulin and glucagon secretion via two receptor subtypes: an in vitro study of pancreatic islets from somatostatin receptor 2 knockout mice. *Endocrinology* 141:111–117, 2000
37. Tulipano G, Soldi D, Bagnasco M, Culler MD, Taylor JE, Cocchi D, Giustina A: Characterization of new selective somatostatin receptor subtype-2 (sst2) antagonists, BIM-23627 and BIM-23454: effects of BIM-23627 on GH release in anesthetized male rats after short-term high-dose dexamethasone treatment. *Endocrinology* 143:1218–1224, 2002