Effects of Cysteamine and Antibody to Somatostatin on Islet Cell Function In Vitro

Evidence that Intracellular Somatostatin Deficiency Augments Insulin and Glucagon Secretion

Yogesh C. Patel, Irene Pierzchala, M. Amherdt, and Lelio Orci

Fraser Laboratories, McGill University, Departments of Medicine, Neurology, and Neurosurgery, Royal Victoria Hospital and Montreal Neurological Institute, Montreal, Quebec H3A 1A1, Canada; and the Institute of Histology and Embryology, Geneva Medical School, Geneva, Switzerland

Abstract

In this study we have characterized the effects of cysteamine (CHS) on the cellular content and release of immunoreactive somatostatin (S-14 LI), insulin (IRI), and glucagon (IRG) from monolayer cultures of neonatal rat islets. Incubation of cultures with 0.1–10 mM CHS for 1 h led to an apparent, dose-dependent reduction of cellular S-14 LI that was 50% of control at 0.3 mM, 87% at 1 mM, and 95% at 10 mM. IRI content was unaffected by CHS up to 1 mM, but at 10 mM 90% loss of IRI occurred. All concentrations were without effect on IRG content. The loss of S-14 LI and IRI was completely reversible with time, but with different recovery rates for the two hormones (48 h for S-14 LI, and 72 h for IRI). Released S-14 LI rose progressively with increasing doses of CHS from 21±2.5 pg/ml per hour to 41±1.4 pg/ml per hour at CHS concentrations of 5 mM and 10 mM. IRI and IRG secretion were both also significantly enhanced (by 55% and 88%, respectively), despite the elevated medium S-14 LI. Since CHS reduced cellular S-14 LI but augmented medium S-14 LI, the relative effects of CHS (1 mM) and immunoneutralization with antibody to S-14 LI on IRI and IRG secretion were tested. Anti S-14 LI alone stimulated basal IRG (67%) but not IRI. Cultures rendered S-14 LI deficient with both CHS and anti-S-14 LI exhibited threefold and 2.3-fold potentiation of IRG and IRI secretions, respectively, greater than that expected from the separate effects of the two agents. Increasing medium glucose from 2.8 mM to 16.7 mM stimulated IRI release by 86% and suppressed IRG by 53%. CHS (1 mM) and anti-S-14 LI further augmented stimulated IRI release, by 30%; although 16.7 mM glucose suppression of IRG was still maintained under these conditions, the quantitative IRG response was significantly greater. These results suggest that CHS induces an apparent loss of islet S-14 LI, and at high doses, of IRI as well, but has no effect on A cells. Complete islet S-14 LI deficiency augments IRI and IRG secretion over a wide range of glucose concentrations, suggesting a physiological role of D cells on B cell and A cell regulation. D cell modulation of B cells requires cellular but not extracellular S-14 LI, being mediated probably through direct intracellular communication, whereas the A cells seem to be regulated by both direct contact as well as through locally secreted S-14 LI.

Introduction

The sulfhydryl drug cysteamine (CHS)[1] or mercaptoethylamine was reported by Selye and Szabo (1) in 1973 to induce severe perforating duodenal ulcers in rats. The ulcerogenic effect of this agent was found to be due to an increase in gastric acid and gastrin secretion and could be prevented by somatostatin administration (2). Subsequent studies by Szabo and Reichlin (3) showed that CHS produced an apparent depletion of somatostatin-14-like immunoreactivity (S-14 LI) in the gastric and duodenal mucosa and thereby induced peptic ulceration probably through abolition of the paracrine inhibitory effects of somatostatin on gastrin and gastric acid secretion. A number of laboratories have now confirmed that CHS administered orally or systemically in single doses evokes a rapid, generalized, and reversible loss of S-14 LI from tissues and plasma (3–8). Cysteamine does not lead to a true loss of somatostatin molecules; it probably acts through a chemical modification of the disulfide bond to mask the antigenic sites in the cyclized segment of S-14, a change that also renders the molecule biologically unreactive (4, 7, 9–11). The biological effects resulting from cysteamine-induced S-14 LI loss can be reversed by exogenous S-14 administration (2, 7).

Considerable indirect evidence suggests that the somatostatin producing D cells of the islets of Langerhans serve as important physiological regulators of A and B cell function (12–19). The effects of somatostatin on the A and B cells are believed to be mediated both through intra-islet regulatory interactions, as well as systemically from small concentrations of the peptide reaching the islet cells via the arterial circulation. Evidence for these concepts derives from such observations as the non-random distribution of D cells in relation to A and B cells in the islets (12), the presence of gap junctions between adjacent islet cells (12), the finding of specific receptors for somatostatin on B, A, and D cells in numbers that correlate closely with the known actions of the peptide on each of the three islet cell types (17), and the demonstration that concentrations of somatostatin comparable to those in the arterial circulation can inhibit insulin and glucagon release (16). Furthermore, electrophysiological studies (19) and the transfer of small molecules from one cell type to another (12–15, 18) strongly

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Address correspondence to Dr. Patel, Room M3-10, Royal Victoria Hospital, 687 Pine Avenue West, Montreal, Quebec H3A 1A1, Canada.

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1. *Abbreviations used in this paper:* CHS, cysteamine; IRG, immunoreactive glucagon; IRI, immunoreactive insulin; NSS, normal sheep serum; S-14 LI, somatostatin-14-like immunoreactivity.

suggest that intercellular communication is an important mechanism for islet cell regulation.

In the absence of a specific antagonist of somatostatin or a means for selective ablation of islet D cells, direct demonstration of a physiological role of somatostatin in the islet has proved difficult. Although antibody to somatostatin has been widely used as a tool for blocking the effects of released somatostatin, such immunoneutralization experiments with intact or isolated mammalian islets have yielded variable and contradictory results probably because of poor or uneven penetration of antibody molecules into the islet interstitial spaces (20–25). The availability of CHS, and especially its use combined with somatostatin antibody on monolayer cultures of islet cells, has afforded a potential means for inducing total islet somatostatin deficiency for investigating A and B cell function in a somatostatin-free environment. In this study we have characterized the in vitro effects of CHS, alone, and in combination with somatostatin antibody, on islet cell function. Specifically, we have (*a*) compared the effects of CHS on the cellular content and release of somatostatin, insulin, and glucagon; (*b*) determined the dose-response characteristics of CHS; (*c*) analysed the molecular forms of S-14 LI inactivated by CHS; (*d*) determined whether CHS exerts a selective action on D cells or whether A and B cells are also affected; (*e*) investigated the effects of CHS-induced islet somatostatin depletion on insulin and glucagon secretion; (*f*) compared the effects of somatostatin deficiency in cells (induced by CHS) and in medium (neutralized with excess somatostatin antibody), on insulin and glucagon secretion.

Methods

Monolayer cultures of islet cells. Monolayer cultures of pancreatic islet cells from 3-d-old Wistar rats were established in 35-mm diam plastic Petri dishes by minor modifications of the technique previously described from our laboratory (26) and used from day 8 to day 12. The culture medium consisted of Eagle's minimum essential medium with Earle's salt base supplemented with 10% heat-inactivated fetal calf serum (Gibco Laboratories, Long Island, NY), penicillin (100 U/ml), streptomycin (100 μg/ml), and fungizone (0.25 μg/ml). The glucose concentration in the medium was 16.7 mM until 24 h before the experiments, when it was decreased to 5.6 mM. For the immunocytochemical localization of somatostatin, insulin, and glucagon positive cells, the indirect immunofluorescence technique was used on islet cells fixed in situ (27). The antisera used for these studies were identical to those employed for radioimmunoassays (RIAs) of the three islet hormones (see below).

Secretion experiments. The incubation medium for the secretion studies consisted of Eagle's minimal essential medium with 0.5% fetal calf serum and 800 μg/ml bacitracin (Sigma Chemical Co., St. Louis, MO). Released islet hormones were stable in this medium under the incubation conditions. CHS (Aldrich Chemical Co., Inc., Milwaukee, WI) was dissolved in sterile double-distilled water to give a stock solution of 100 mM, and the pH was adjusted to 7.4 with 1 N NaOH. Suitable volumes of the stock were subdiluted in the incubation medium to give the desired concentrations. Before conducting the secretion experiments, the Petri dishes were randomly divided into groups of five, and the maintenance media were discarded and replaced with 1 ml control medium or medium containing test substances (CHS, high or low glucose, sheep anti-somatostatin serum, and non-immune sheep serum). Experiments with cysteamine alone were terminated after 1 h, whereas experiments using both CHS and antibody were incubated for 3 h. Media at the end of each incubation were collected and centrifuged (500 *g* for 10 min) to remove desquamated cells, and the supernatant was stored at −20°C for subsequent assays. The cells were scraped in 1 ml 1 N acetic acid at 0°C, the islet hormones were extracted by sonication and boiling (28), the extract centrifuged (1,000 *g* for 10 min), and the supernatant was kept at −20°C for RIA.

Neutralization experiments with anti-somatostatin antibody. Immunoneutralization of released somatostatin was accomplished by including in the incubation medium 10 μl/ml of a potent sheep antiserum against S-14 LI (binding capacity, 10 μg S-14/ml) (29), or an equivalent volume of normal sheep serum (NSS). This amount of antibody was calculated to be 1,000 times in excess of the quantity required to neutralize the basally released S-14 LI. The presence of excess antibody in the medium was confirmed for each experiment by reacting 0.1 ml of the antibody containing medium from each dish before and after exposure to the cultures with 5 pg [^{125}I-Tyr]-S-14 for 24 h at 4°C. Mean binding capacities of 81±1% (before) and 82±0.5% (after) were obtained.

RIAs. Somatostatin-like immunoreactivity in the medium and in cell extracts was measured as S-14 LI by RIA using rabbit antibody R149 (directed towards the central segment of S-14, which detects S-14 and somatostatin-28 [S-28] equally), [^{125}I-Tyr]-S-14, and S-14 standards (28). Assay sensitivity was 0.7 pg S-14. Immunoreactive insulin (IRI) was measured by RIA using Wright guinea pig antiporcine insulin serum, ^{125}I-porcine insulin, and rat insulin standards (Novo Industrie, Denmark) (26). Assay sensitivity was 20 pg. Immunoreactive glucagon (IRG) was measured by RIA using rabbit antibody 30K (R. H. Unger, Dallas, TX), ^{125}I-porcine glucagon, and porcine glucagon standards (Novo Industrie, Denmark) (26). Assay sensitivity was 3 pg. The nonspecific effect of cysteamine on antibody binding of radioligand was excluded for each of the three RIAs by incubating medium containing 1.0 mM and 10 mM cysteamine in the RIAs. These concentrations of cysteamine were without effect on radioligand binding of antibody in each of the three assay systems. Likewise, cysteamine (10 mM) did not reduce the binding capacity of the sheep anti-S-14 LI serum when incubated with it in the immunoneutralization experiments.

Gel chromatography. The molecular forms of S-14 LI in cell extracts were characterized by gel chromatography (30). A 70 × 1-cm column of Sephadex G-50 (superfine) was eluted with 6 M urea, 50 mM PO_4, pH 7.5. S-14 LI in the 1-ml fractions was determined by RIA. The elution positions of S-14 and S-28 were determined by precalibration of the columns with 1–2 ng synthetic peptide eluted with 6 M urea, 50 mM PO_4, and measured as S-14 LI by RIA of the column filtrate.

Statistical analysis. Data were analysed by analysis of variance as applied to multifactorial experiments with unequal cell frequencies (31). The unpaired Student's *t* test was used for comparing the recovery of S-14 LI and IRI between CHS and control cultures.

Results

Effect of CHS on cellular content of somatostatin, insulin, and glucagon. Fig. 1 shows the effect of increasing concentrations of CHS on islet hormone content. CHS induced a severe, dose-dependent depletion of islet S-14 LI, which was 50% of control at 0.3 mM, 87% at 1 mM, and 95% at 10 mM. The drug was without effect on islet IRI content up to a concentration of 1 mM. Higher concentrations led to a progressive loss of islet IRI content, which reached 11% of control values at 10 mM. All concentrations of CHS tested were without effect on islet IRG content. Islet S-14 LI depletion was completely reversible with time. In the experiment shown in Fig. 2, cellular S-14 LI was determined daily for 4 d in groups of Petri dishes initially incubated for 1 h with either 1 mM CHS or control

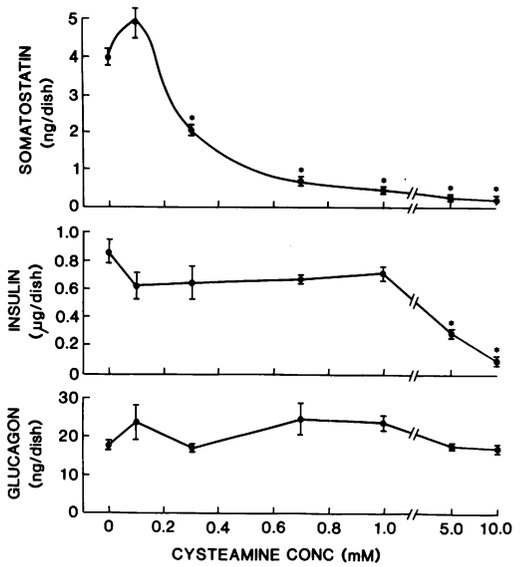

Figure 1. Effect of graded concentrations (CONC) of CHS on intracellular content of somatostatin, insulin, and glucagon in monolayer islet cell cultures (n = 5 for each point; mean±SE; $P < 0.01$).

medium. CHS-induced loss of islet S-14 LI underwent spontaneous recovery to 75% of control values by 24 h and complete recovery thereafter. In a similar but separate experiment, the recovery of IRI after exposure of the cultures to 10 mM CHS was evaluated (Table I). Like S-14 LI, cellular IRI also underwent spontaneous recovery but with a more prolonged time course. Thus, at 24 h when S-14 LI was nearly repleted (Fig. 2), IRI was still reduced by 70%. Recovery of IRI to near control values occurred by 48–72 h.

To determine which molecular forms of S-14 LI were affected by CHS, extracts of control cultures or cultures exposed to CHS 1 mM for 1 h were subjected to Sephadex G-50 chromatography. 98% of S-14 LI in control cultures coeluted with synthetic S-14; the remaining 2% corresponding to a 14-kD prosomatostatin form (Fig. 3). Residual S-14 LI in CHS-treated cultures was also predominantly S-14, but the relative amount of prosomatostatin (9%) was much higher than in

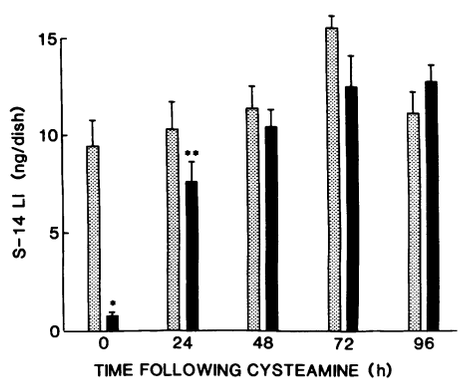

Figure 2. Recovery of cellular S-14 LI in groups of five Petri dishes after incubation for 1 h with 1 mM CHS compared with cultures incubated with control medium. ▭, control; ■, post-CHS, *, $P < 0.01$; **, $P < 0.05$.

Table I. Recovery of Cellular IRI Content of Cultures Exposed to 10 mM CHS

Time after exposure to CHS or control medium	Cellular IRI content	
	Control	CHS
h	*ng/dish*	
1	576±45	147±15*
10	698±80	319±35*
24	919±160	298±20*
48	693±35	574±60
72	684±55	612±15

* $P < 0.01$ (n = 4).

control cultures. In absolute terms, virtually all the S-14 LI lost as a result of CHS could be accounted for by the S-14 molecular species.

Morphology. Islet cell clusters remained morphologically unchanged as assessed by light microscopy during and after exposure to CHS. By immunofluorescence, there was no noticeable difference between the B, A, and D cells in control and CHS-treated cultures. In particular, the somatostatin and insulin positive cells, after exposure to CHS (1 mM and 10 mM), exhibited the same intensity of fluorescence as control cells, despite the severe reduction in S-14 LI and IRI in cell extracts as determined by RIA.

Effect of CHS on basal release of somatostatin, insulin, and glucagon. Fig. 4 depicts the levels of S-14 LI, IRI, and

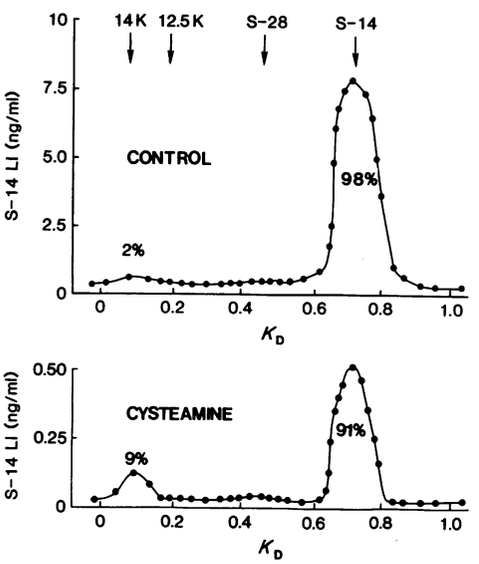

Figure 3. Gel chromatographic characterization of the molecular forms of S-14 LI in extracts of control islet cell cultures or cultures exposed to CHS (1 mM) for 1 h. A 1 × 70 cm Sephadex G-50 (superfine) column was equilibrated and eluted with 6 M urea, 50 mM PO_4 buffer, 7.5. S-14 LI in the column filtrate was measured by RIA. The column was precalibrated with markers of known molecular weight including somatostatin-14 (S-14, 1 ng) and somatostatin-28 (S-28, 1 ng), both of which were detected by RIA. K_D 0 = void volume; K_D 1 = salt volume.

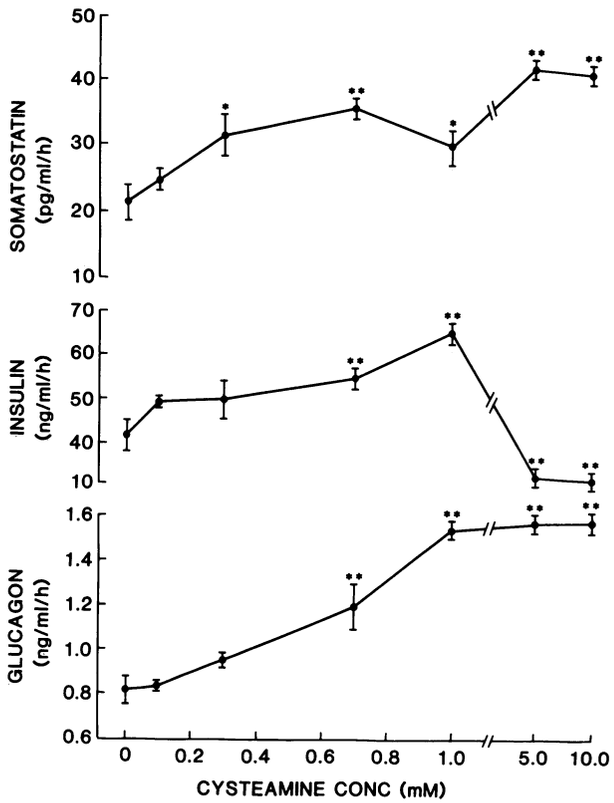

Figure 4. Effect of graded concentrations (CONC) of CHS on the release of somatostatin, insulin, and glucagon from monolayer islet cell cultures. Medium glucose concentration, 5.5 mM (n = 5 for each point). Mean±SE. *, $P < 0.05$; **, $P < 0.01$.

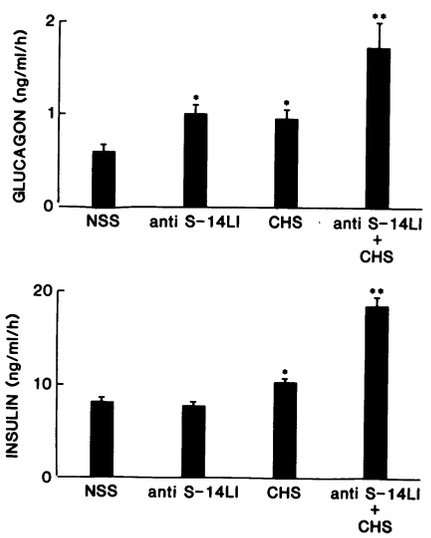

Figure 5. Comparison of the effects of CHS (1 mM) and a sheep somatostatin antibody (anti-S-14 LI) alone and in combination on glucagon and insulin release from monolayer islet cell cultures. Medium glucose concentration, 5.5 mM. NSS = control incubations with nonimmune sheep serum (n = 5 for each incubation condition) *, $P < 0.05$ vs. NSS; **, $P < 0.01$ vs. anti-S-14 LI or CHS.

IRG in the medium from the CHS dose-response experiment shown in Fig. 1. Released S-14 LI rose progressively with increasing doses of CHS from a control value of 21±2.5 pg/ml per hour to 41±1.4 pg/ml per hour at CHS concentrations of 5 mM and 10 mM. Secretion of IRI was also enhanced from 42±3.8 ng/ml per hour to 65±2.4 ng/ml per hour for a CHS concentration of 1 mM. Higher doses of CHS led to a dramatic fall in medium IRI levels concomitant with the loss of intracellular IRI. CHS in concentrations up to 1 mM led to a dose-dependent increase in IRG secretion, which rose from a control value of 0.8±0.06 ng/ml per hour to 1.5±0.045 ng/ml per hour. Higher doses (5 mM, 10 mM) did not produce any additional effect on IRG output.

Effect of CHS and somatostatin antibody on basal insulin and glucagon secretion. Since CHS depleted cellular S-14 LI content but increased medium S-14 LI, the next set of experiments (Fig. 5) was performed to compare the relative effects on basal insulin and glucagon release of either CHS alone or CHS + anti-S-14 LI serum to produce a deficiency of S-14 LI in both cells and in medium. Basal IRG secretion from the cultures increased by 67% from 0.6±0.05 ng/ml per hour to 1±0.1 ng/ml per hour ($P < 0.05$) in the presence of excess sheep anti-S-14 LI antibody compared with nonimmune sheep serum (NSS). 1 mM CHS also significantly enhanced basal IRG secretion as shown earlier (Fig. 1) to about the same extent (by 57%) as with S-14 LI antibody. Cultures rendered S-14 LI deficient with both CHS and antibody exhibited potentiation of IRG secretion from 0.6±0.05 ng/ml per hour to 1.75±0.26 ng/ml per hour, a response nearly three times greater (186% increase) than with either agent alone.

In the case of IRI secretion, CHS produced the expected increase in basal release (10.5±0.5 ng/ml per hour compared with 8.2±0.5 ng/ml per hour) ($P < 0.05$), whereas S-14 LI antibody was ineffective. The combined use of antibody and CHS evoked a marked 2.3-fold increase in IRI secretion to a level of 18.6±2 ng/ml per hour, much greater than that expected from the separate effects of these two agents.

Effect of glucose on insulin and glucagon secretion in the presence of CHS and somatostatin antibody. In this set of experiments the effect of glucose on B and A cell function in cultures exposed to 1 mM CHS or CHS and S-14 LI antibody was examined (Fig. 6 and Table I). At a glucose concentration of 2.8 mM, IRI and IRG secretion were both stimulated by CHS and further stimulated by CHS and anti-S-14 LI. These findings are analogous to those obtained in Fig. 5 with 5.5 mM glucose. Increasing the glucose concentration to 16.7 mM led to an 86% increase in IRI secretion. In the presence of CHS and CHS + anti-S-14 LI, glucose-stimulated IRI secretion was further augmented compared with the 16.7 mM glucose control. Maximum stimulation, however, was observed with CHS alone, and although CHS + anti-S-14 LI evoked an IRI response significantly greater than with 16.7 mM glucose alone, the combined effect of the two agents was paradoxically lower than with CHS itself. Increasing the glucose concentration from 2.8 mM to 16.7 mM resulted in significant suppression of IRG for all three test conditions investigated. Although the percent suppression of IRG by high glucose was comparable for the three conditions, the actual output of IRG from the glucose suppressed A cells was significantly greater in the presence of CHS and greater again with CHS and anti-S-14 LI than in the absence of these two agents.

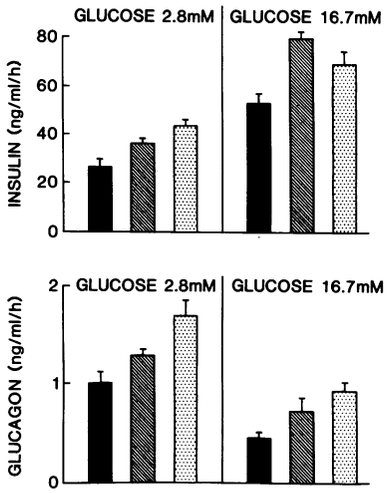

Figure 6. Effect of glucose (2.8 mM, 16.7 mM) on insulin and glucagon secretion from monolayer islet cell cultures in the presence of CHS (1 mM) and CHS + somatostatin antibody (anti-S-14 LI). ▪, control; ▪, CHS plus nonimmune sheep serum; ▫, cysteamine plus anti-S-14 LI. Results of statistical comparisons between the different responses are summarized in Table II. (n = 4 for each incubation condition).

Discussion

Since the first demonstration that CHS depletes S-14 LI from tissues and plasma (3), there has been a steady proliferation of reports characterizing the detailed endocrine effects of this drug (4–11, 32, 33). To date, only S-14 LI and prolactin have been reported to be susceptible to CHS out of a wide range of peptide and polypeptide hormones tested. Based on the present findings, IRI is also depleted by CHS but at doses at least ten times greater than those required for S-14 LI and prolactin (32).

The mechanism of action of CHS on somatostatin has not been completely elucidated. It has been suggested that CHS might act by accelerating the intracellular degradation of somatostatin, thereby inducing a true depletion of S-14 LI (3), or more likely, through a chemical modification of the disulphide bond, which would render the molecule immunologically and biologically unreactive (4, 9). CHS is ineffective when incubated directly with synthetic S-14 or S-28 (4, 9), suggesting that its action requires the intracellular milieu and does not represent a simple reduction of the disulphide bond. In the case of S-28 (which is cyclized in the COOH-terminal half of the molecule and linear in the NH_2-terminal half), our recent finding that CHS induces a loss of tissue S-28 when measured as $S\text{-}28_{[15-28]}$ LI but not when assessed as $S\text{-}28_{[1-14]}$ LI, provides strong evidence that the drug acts on the 15–28 segment of the S-28 molecule and renders it nonimmunoreactive, probably through an interaction with the disulphide bond. This mechanism probably also accounts for CHS-induced S-14 loss. These findings strongly suggest that CHS-induced "depletion" of somatostatin is not a true depletion of somatostatin molecules but rather an apparent depletion due to masking of antigenic sites. Our inability to detect a noticeable reduction in S-14 LI by immunofluorescence after CHS treatment of the cultures is of interest. It could be due to the relative insensitivity of the immunofluorescence technique compared with RIA, although more detailed studies (to be reported separately) using quantitative electron microscopic immunocytochemistry with the protein-A gold technique have shown identical numbers of S-14 antigenic sites in D cells before and after CHS (Patel, Y. C., M. Ravazzola, and L. Orci. Manuscript in preparation). This suggests that the S-14 antigenic sites in the CHS-modified somatostatin molecule are exposed as a result of tissue fixation, whereas in the soluble form (e.g., in tissue extracts) the antigenic sites are masked.

S-14 LI in cultured islet cells, as in the whole rat pancreas, consists almost entirely of the S-14 molecular species. From our gel chromatographic analyses of S-14 LI in CHS-treated islet cells, it is evident that S-14 is the main molecular form inactivated by CHS. Since the cultures contained virtually no S-28 equivalent material, the effect of CHS on S-28 cannot be determined from this study. Our finding of a relative accumulation of prosomatostatin in CHS-exposed cultures (from 2% to 9% of total S-14 LI) is of interest and suggests increased synthesis and/or impaired post-translational enzymatic pro-

Table II. Effect of Glucose on Insulin and Glucagon Secretion in the Presence of CHS and Somatostatin Antibody

Experiment	Incubation condition	IRI release*		IRG release*	
		ng/ml per hour	*P*	*ng/ml per hour*	*P*
1	glucose 2.8 mM	29.7±2	—	1.05±0.1	—
2	glucose 2.8 mM + CHS (1 mM) + NSS	37±0.5	<0.5 (vs. 1)	1.2±0.04	<0.05 (vs. 1)
3	glucose 2.8 mM + CHS (1 mM) + anti-S-14 LI	43.6±0.9	<0.01 (vs. 1) NS (vs. 2)	1.72±0.14	<0.001 (vs. 1) <0.01 (vs. 2)
4	glucose 16.7 mM	53.7±3	<0.001 (vs. 1)	0.46±0.05	<0.01 (vs. 1)
5	glucose 16.7 mM + CHS (1 mM) + NSS	79.4±1.8	<0.001 (vs. 2) <0.001 (vs. 5)	0.73±0.14	<0.05 (vs. 2) NS (vs. 4)
6	glucose 16.7 mM + CHS (1 mM) + anti-S-14 LI	69±4.8	<0.01 (vs. 3) <0.01 (vs. 4) <0.05 (vs. 5)	0.97±0.08	<0.001 (vs. 3) <0.01 (vs. 4) NS (vs. 5)

* Mean data±SE (n = 4).

cessing of prosomatostatin to S-14 and S-28. CHS-induced loss of S-14 LI and IRI underwent spontaneous and complete recovery, but with a different time course, the recovery of S-14 LI being more rapid than that of IRI. Whether the reappearance of S-14 LI and IRI in cells represents newly synthesized immunoreactivity or reversal of the chemical reaction between CHS and S-14 LI or IRI is not known.

One of the salient findings of this study was the demonstration that CHS-induced depletion of S-14 LI was associated with increased secretion of S-14 LI, IRI, and IRG. The increased basal accumulation of S-14 LI in the medium in the face of decreasing intracellular stores is unusual and suggests a very rapid rate of secretion of residual cellular immunoreactivity. Despite the elevated basal output of S-14 LI, the secretion of IRI and IRG was stimulated by CHS. Neutralization of medium S-14 LI with antibody further augmented basal IRG and IRI secretion, although the antibody alone was effective in stimulating only A cell secretion. The additive effect of antibody and CHS on IRI secretion can probably be accounted for by secondary stimulation of B cells by IRG. These experiments suggest that total islet S-14 LI deficiency (induced by CHS and antibody) augments the basal release of both IRI and IRG.

Like the basal release, glucose stimulated IRI release was also augmented by CHS, although the combination of CHS and anti-S-14 LI produced no additive effect, and in fact a slightly lower response than CHS alone for reasons that are not clear. Glucagon showed the expected suppression with high glucose. While glucose-induced suppression of A cells also occurred in the absence of S-14 LI (with CHS or CHS plus antibody), the level of suppression of the A cells was less than under control conditions (i.e., when D cell function was preserved). From these experiments it can be concluded that both A and B cells hyperfunction basally as well as in the presence of high glucose when isolated from D cell inhibition. Thus, somatostatin does not alter the qualitative response of the A and B cells to glucose, but it clearly modulates the quantitative response. These postulated effects are in keeping with increasing evidence that B, A, and D cells of the islets of Langerhans may regulate the secretory functions of neighboring cells by direct local interactions (12–19). Such interactions could be mediated by a paracrine process (thought to occur when a substance secreted by a cell into the interstitial space influences the function of an adjacent cell), and/or through gap junctional coupling, allowing direct cell to cell communication. For example, the finding that intracellular S-14 LI depletion is accompanied by stimulation of IRI and IRG secretion even in the presence of elevated extracellular concentrations of S-14 LI (Fig. 4) clearly implies a direct action of D cells on the A and B cells. The dissociated effects of S-14 LI antibody and CHS on IRI secretion is more difficult to interpret but suggests that D cell modulation of B cells may occur primarily through direct intercellular communication, whereas the A cells may be regulated by direct contact with D cells as well as through locally secreted S-14.

Only one other study has previously investigated the effects of CHS on pancreatic endocrine function with conclusions somewhat different from ours. Sorenson et al. (8) studied the isolated perfused pancreas of rats 24 h after a single subcutaneous injection of CHS and found a significant decrease in pancreatic S-14 LI release but no increase in the basal or glucose stimulated IRI or IRG responses (indeed, a significant reduction in basal IRG output was found). These differences are difficult to explain but perhaps can be accounted for by the very dissimilar model systems, as well as differences in the dose of CHS, route of administration, and the time interval after CHS before the experiments were performed. The use of immunoneutralization alone for assessing a functional role of endogenous somatostatin on insulin and glucagon secretion from mammalian islets has been described from several laboratories, with conflicting results (20–25, 34). Passive immunization with anti-S-14 LI serum using whole animals or the isolated perfused pancreas has generally shown no direct effect on IRI or IRG secretion because of poor penetration of antibody molecules into intact islets (20–22). Isolated islets exposed to anti-S-14 gamma globulin have been reported by Itoh et al. (25) to secrete increased quantities of IRI and IRG. Other studies, however, also using isolated islets or monolayer cultures of islets, have found no effect of anti-S-14 LI on IRI secretion (23) or on IRG secretion (34).

These reports highlight the inadequacies of the approaches used so far for elucidating direct local effects of somatostatin on A and B cells. As illustrated by our findings, the availability of an agent such as CHS, which produces rapid, potent, and reversible loss of somatostatin from cells, clearly represents a powerful new tool for defining the physiological role of islet D cells. Furthermore, the use of graded concentrations of CHS for inducing selective somatostatin deficiency and combined somatostatin and insulin deficiency, promises newer insights into the function of not only D cells but the other islet cells as well.

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References

1. Selye, H., and S. Szabo. 1973. Experimental model for production of perforating duodenal ulcers by cysteamine in the rat. *Nature (Lond.)* 244:458–459.

2. Schwedes, U., K. Usadel, and S. Szabo. 1977. Somatostatin prevents cysteamine-induced duodenal ulcer. *Eur. J. Pharmacol.* 44: 195–196.

3. Szabo, S., and S. Reichlin. 1981. Somatostatin in rat tissues is depleted by cysteamine administration. *Endocrinology.* 109:2255–2257.

4. Sagar, S. M., D. Landry, W. J. Millard, T. M. Badger, M. A. Arnold, and J. B. Martin. 1982. Depletion of somatostatin-like immunoreactivity in the rat central nervous system by cysteamine. *J. Neurosci.* 2:225–231.

5. Sagar, S. M., and J. B. Martin. 1982. The effect of cysteamine on immunoreactive somatostatin in the rabbit retina. *Neurosci. Lett.* 34:265–269.

6. Palkovits, M., M. J. Brownstein, L. E. Eiden, M. C. Beinfeld, J. Russell, A. Arimura, and S. Szabo. 1982. Selective depletion of somatostatin in rat brain by cysteamine. *Brain Res.* 240:178–180.

7. Brown, M. R., L. A. Fisher, P. E. Sawchenko, L. W. Swanson, and W. W. Vale. 1983. Biological effects of cysteamine: relationship to somatostatin depletion. *Regul. Pep.* 5:163–179.

8. Sorenson, R. L., L. H. Grouse, and R. P. Elde. 1983. Cysteamine

blocks somatostatin secretion without altering the course of insulin or glucagon release. *Diabetes.* 32:377–379.

9. Patel, Y. C., and I. Pierzchala. 1985. Cysteamine induces a loss of tissue somatostatin-28 when measured as somatostatin-$28_{[15-28]}$ like immunoreactivity but not when assessed as somatostatin-$28_{[1-14]}$ like immunoreactivity: evidence for the importance of the disulfide bond for cysteamine action. *Endocrinology.* In press.

10. Scammel, J. G., and P. S. Dannies. 1984. Depletion of pituitary prolactin by cysteamine is due to loss of immunologic activity. *Endocrinology.* 114:712–716.

11. Millard, W. J., S. M. Sagar, J. W. Simpkins, R. E. Owens, T. M. Badger, H. G. Friesen, and J. B. Martin. 1983. Cysteamine induces depletion of both immunological and biological prolactin activity in the anterior pituitary and blood of the rat. *Endocrinology.* 113:2161–2167.

12. Orci, L. 1982. Macro- and micro-domains in the endocrine pancreas. *Diabetes.* 31:538–565.

13. Michaels, R. L., and J. D. Sheridan. 1981. Islets of Langerhans: dye coupling among immunocytochemically distinct cell types. *Science (Wash. DC).* 214:801–803.

14. Meda, P., E. Kohen, C. Kohen, A. Rabinovitch, and L. Orci. 1982. Direct communication of homologous and heterologous endocrine islet cells in culture. *J. Cell. Biol.* 92:221–226.

15. Kohen, E., C. Kohen, and A. Rabinovitch. 1982. Cell-to-cell communication in rat pancreatic islet monolayer cultures is modulated by agents affecting islet-cell secretory activity. *Diabetes.* 32:95–98.

16. Kawai, K., E. Ipp, L. Orci, A. Perrelet, and R. H. Unger. 1982. Circulating somatostatin acts on the islets of Langerhans by way of a somatostatin-poor compartment. *Science (Wash. DC).* 218:477–478.

17. Patel, Y. C., M. Amherdt, and L. Orci. 1982. Quantitative electron microscopic autoradiography of insulin, glucagon, and somatostatin binding sites on islets. *Science (Wash. DC).* 217:1155–1156.

18. Kohen, E., C. Kohen, B. Thorell, D. H. Mintz, and A. Rabinovitch. 1979. Intercellular communication in pancreatic islet monolayer cultures: a microfluorometric study. *Science (Wash. DC).* 204:862–865.

19. Pace, C. S., and J. T. Tarvin. 1981. Somatostatin: mechanism of action in pancreatic islet B-cells. *Diabetes.* 30:836–842.

20. Tannenbaum, G. S., J. Epelbaum, E. Colle, P. Brazeau, and J. B. Martin. 1978. Antiserum to somatostatin reverses starvation-induced inhibition of growth hormone but not insulin secretion. *Endocrinology.* 102:1909–1914.

21. Schusdziarra, V., E. Zyznar, D. Rouiller, G. Boden, J. C. Brown, A. Arimura, and R. H. Unger. 1980. Splanchnic somatostatin: a hormonal regulator of nutrient homeostasis. *Science (Wash. DC).* 207:530–532.

22. Sorenson, R. L., D. V. Lindell, and R. P. Elde. 1980. Glucose stimulation of somatostatin and insulin release from the isolated, perfused rat pancreas. *Diabetes.* 29:747–751.

23. Barden, N., J.-P. Cote, M. Lavoie, and A. Dupont. 1978. Secretion of somatostatin by rat islets of Langerhans and gastric mucosa and a role for pancreatic somatostatin in the regulation of glucagon release. *Metabolism.* 27:1215–1218.

24. Taniguchi, H., M. Utsumi, M. Hasegawa, T. Kobayashi, Y. Watanabe, K. Murakami, M. Seki, A. Tsutou, H. Makimura, M. Sakoda, and S. Baba. 1977. Physiologic role of somatostatin-insulin release from rat islets treated by somatostatin anti-serum. *Diabetes.* 26:700–702.

25. Itoh, M., L. Mandarino, and J. E. Gerich. 1980. Antisomatostatin gamma 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.

26. Patel, Y. C., M. Amherdt, and L. Orci. 1979. Somatostatin secretion from monolayer cultures of neonatal rat pancreas. *Endocrinology.* 104:676–679.

27. Rufener, C., M. Amherdt, M. P. Dubois, and L. Orci. 1975. Ultrastructural immunocytochemical localization of somatostatin in rat pancreatic monolayer culture. *J. Histochem. Cytochem.* 23:866–869.

28. Patel, Y. C., and S. Reichlin. 1978. Somatostatin in hypothalamus, extrahypothalamic brain and peripheral tissues of the rat. *Endocrinology.* 102:523–530.

29. Rorstad, O. P., J. Epelbaum, P. Brazeau, and J. B. Martin. 1979. Chromatographic and biological properties of immunoreactive somatostatin in hypothalamic and extrahypothalamic brain regions of the rat. *Endocrinology.* 105:1083–1092.

30. Patel, Y. C., T. Wheatley, and C. Ning. 1981. Multiple forms of immunoreactive somatostatin: characterization and comparison of distribution in neural and non-neural tissues and portal plasma of the rat. *Endocrinology.* 109:1943–1949.

31. Winer, B. J. 1971. Statistical Principles in Experimental Design. McGraw-Hill Book Co., New York. Second ed. 907 pp.

32. Millard, W. J., S. M. Sagar, D. M. D. Landis, and J. B. Martin. 1982. Cysteamine: a potent and specific depletor of pituitary prolactin. *Science (Wash. DC).* 217:452–454.

33. Srikant, C. B., and Y. C. Patel. 1984. Cysteamine-induced depletion of brain somatostatin is associated with upregulation of cerebrocortical somatostatin receptors. *Endocrinology.* 115:990–995.

34. Fujimoto, W. Y., S. Kawazu, M. Ikeuchi, and Y. Kanazawa. 1983. In vitro paracrine regulation of islet B-cell function by A and D-cells. *Life Sci.* 32:1873–1878.