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Sensitivity of rat pancreatic A and B cells to somatostatin*

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Summary. Islet A and B cells were purified from the rat pancreas and examined for their respective sensitivity to somatostatin. Both somatostatin-14 (S14) and -28 (S28) inhibited glucagon and insulin release through direct interactions with the corresponding cell types. A dose-dependent suppression of the secretory activities was paralleled by a reduction in cellular cyclic AMP formation with similar ED_{50} values for both actions. The somatostatin effects on pancreatic hormone release may thus be mediated via an inhibition of adenylate cyclase activity. In pancreatic A cells, S14 and S28 were equally potent inhibitors with ED_{50} values ranging from 2×10^{-12} to 2×10^{-11} mol/l. Pancreatic B cells exhibited a similar sensitivity to S28 as the A cells (ED_{50} of 2 to 5×10^{-11} mol/l), but not to S14 (ED_{50} of 2×10^{-9} mol/l). Extrapolation of these *in vitro* sensitivities of islet A and B cells to the *in vivo* situation suggests that both cell types can respond to circulating S28 levels and that A cells are sensitive to both locally and distally released S14. Islet B cells appear insensitive to the normal peripheral S14 levels but could respond to locally released somatostatin. The marked difference in the sensitivities of islet A and B cells to S14 suggests that these cell types are equipped with different somatostatin receptors. This notion was further supported by the cell-selective actions of the synthetic S14 analogues [D-Trp^8, D-Cys^{14}]S14 and desAsn^5[D-Trp^8, D-Ser^{13}]S14.

Key words: Somatostatin, somatostatin analogues, insulin release, glucagon release, cyclic AMP, purified islet cells, pancreatic islets.

Somatostatin immunoreactive cells have been described in various sites of the body [1–6]; their hormonal products are synthesised as preprosomatostatin, a 12,000–14,000 dalton prohormone [7, 8] and released as somatostatin-28 (S28) or somatostatin-14 (S14) [9–12]. The two latter peptides have been identified in the circulation and their administration *in vivo* and *in vitro* leads to the inhibition of a wide variety of physiologic processes [for review, see ref. 13]. In several cases, the specific action of each peptide could not be elucidated as it was difficult to distinguish direct from indirect interactions and to weigh the influence of the administered hormone versus that of the locally released one. In the endocrine pancreas, for example, addition of S14 or S28 leads to inhibition of insulin and glucagon release, with apparently differential effects of the two peptides [14–17]. It remains, however, unknown whether the two somatostatins act directly and independently of each other on both pancreatic A and B cells, whether the somatostatin-induced effects on one cell type influence the secretory function of the other, and whether locally released somatostatin – originating from the islet D cells – participates in the final response. One way to clarify somewhat this complex issue consists in examining the effects of S14, of S28 and of related peptides on purified pancreatic A and B cells.

Materials and methods

Materials

Porcine glucagon was purchased from Novo Industries (Bagsvaerd, Denmark); S14 and its analogues, desAsn^5[D-Trp^8, D-Ser^{13}]S14 and [D-Trp^8, D-Cys^{14}]S14 were obtained from UCB Bioproducts (Braine L'Alleud, Belgium). The purity of the S14 analogues was higher than 98% on TLC and HPLC (UCB product information). S28 came from Sigma (St Louis, Mo, USA). The lyophilised peptides were dissolved in 2 mol/l acetic acid supplemented with 0.25% (wt/vol) bovine serum albumin (BSA; Boehringer-Mannheim, Mannheim, FRG), aliquoted and stored at −80 °C. The phosphodiesterase inhibitor Ro 20-1724 was kindly provided by Hoffman-La Roche (Basel, Switzerland).

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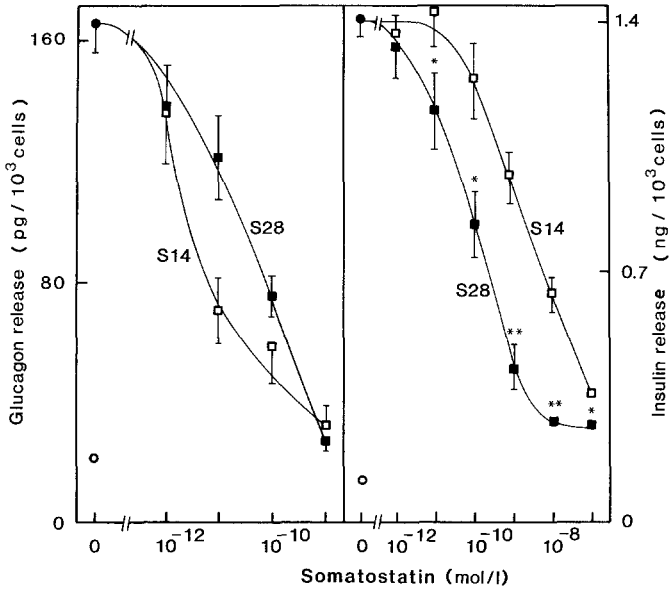

Fig. 1. Effect of somatostatin on glucagon and insulin release from purified A cells (left panel) and purified B cells (right panel). The cells were incubated for 15 min with or without somatostatin (S14: □ S28: ■). For A cells, the medium was supplemented with alanine, arginine and glutamine (2 mmol/l each) with 1.4 mmol/l glucose and 10^{-6} mol/l noradrenaline; for B cells, it contained 20 mmol/l glucose plus 10^{-8} mol/l glucagon. The phosphodiesterase inhibitor Ro 20-1724 was added at min 10 (final concentration 100 μmol/l). ○ basal control (EH with 1.4 mmol/l glucose); ● stimulus control (no somatostatin added). Data represent mean values ± SEM of 5 experiments. The statistical significance of differences between effects of equimolar S14- and S28 concentrations was calculated by the paired Student's t-test. *: $p<0.05$; **: $p<0.001$

Purification of pancreatic A and B cells

The techniques for the preparation of islet A and B cells have been described in full detail elsewhere [18-20]. Briefly, pancreatic islets were isolated from male adult Wistar rats using a modified collagenase technique [19]. The isolated islets were dissociated in calcium-free medium containing 1 mmol/l ethyleneglycol-bis-(β-aminoethyl ether)N,N′-tetraacetic acid (EGTA), 25 μg/ml trypsin (Boehringer-Mannheim) and 2 μg/ml DN-ase (Boehringer) [18]. The dissociated cells were separated by autofluorescence-activated cell sorting into pure A and pure B cell preparations [19, 20]. The isolated cells were re-aggregated for 2 h in a rotatory shaking incubator (Braun, Melsungen, FRG) and then statically cultured for 20 h. The tissue culture medium was CMRL-1066 (GIBCO, Glasgow, UK) supplemented with 10% (vol/vol) heat-inactivated fetal calf serum (GIBCO), 2 mmol/l L-glutamine, penicillin (0.1 mg/ml) and streptomycin (0.1 mg/ml).

Incubations with S14, S28 and S14-analogues

At the end of 20 h culture, the A and B cell aggregates were collected from the petri dishes and washed with Earle's HEPES medium (EH) containing 1.4 mmol/l glucose and 0.5% BSA. The composition of EH has been previously reported [19]. Cell viability as measured with neutral red [21], exceeded 90% in all experiments. Groups of 5×10^4 cells were distributed as 0.1 ml samples over 5 ml plastic tubes (Falcon, Oxnard, Calif., USA) containing 1.4 ml EH. The cells were allowed to sediment for 20 min, before 1 ml of supernatant was carefully aspirated. The remaining 0.5 ml, containing the sedimented cell aggregates, was mixed with an additional volume of 0.5 ml composed as to obtain the final experimental conditions for testing somatostatin (analogues). As it was the purpose to compare the inhibitory effects of the test substances, conditions were selected wherein the islet cells were stimulated through a synergistic interaction of nutrient- and adenylcyclase-dependent regulatory units [22-25]. Thus, pancreatic A cells were incubated at 1.4 mmol/l glucose supplemented with a mixture of amino acids (alanine, arginine and glutamine, 2 mmol/l each) and noradrenaline (10^{-6} mol/l) [22, 24], whereas pancreatic B cells were examined at 20 mmol/l glucose and 10^{-8} mol/l glucagon [23-25]. The somatostatin (analogues) were administered at varying concentration, immediately before start of the 15 min incubation at 37 °C. At the end of incubation, the tubes were centrifuged (1 min - 250 g - 4° C), the supernatant fractions aspirated and analysed for their insulin or glucagon content and the cell pellets resuspended in 0.5 ml 6% trichloroacetic acid (TCA) in order to extract cellular cyclic AMP [25]. The phosphodiesterase inhibitor Ro 20-1724 was added during the last 5 min of incubation in order to create the conditions for measuring the rate of cyclic AMP formation in parallel to the insulin or glucagon release.

Insulin, glucagon and cyclic AMP assays

Cyclic AMP was measured as described previously [25]. The TCA supernatant fraction was extracted four times with 4 ml water-saturated diethylether, lyophilised, and redissolved in 0.05 mol/l sodium acetate buffer (pH 6). The cyclic AMP content of the samples was measured after acetylation with a commercially available [^{125}I]-cyclic AMP radio immuno assay kit (Amersham, Bucks, UK) and is expressed as femtomole per 10^3 cells (fmol/10^3 cells). Insulin and glucagon were measured by radio immuno assay using HPLC-purified [^{125}I]-insulin and [^{125}I]-glucagon tracers [26].

Statistical analysis

Data represent mean values ± SEM of at least four independent experiments. The statistical significance of differences between control and experimental conditions was calculated with two-tailed Student's t-tests. The acceptance level of significance ($p<0.05$) was corrected with the Bonferroni method in case of multiple comparisons [27]. ED_{50} values were calculated with a logit approach based on normal distribution and least square regression analysis [28] and compared with a modified t-test [29].

Results

Effect of S14 and S28 on hormone release from pancreatic A and B cells

Both S14 and S28 exerted a potent inhibition on glucagon release induced by a mixture of amino acids and noradrenaline (Fig. 1, left panel). Their effect was already observed at 10^{-11} mol/l ($p<0.05$); complete suppression was obtained at 10^{-9} mol/l.

The two peptides were also inhibitory to insulin release induced by a mixture of glucose and glucagon (Fig. 1, right panel). This effect became statistically significant at 10^{-11} mol/l for S28 ($p<0.05$) and at 10^{-9} mol/l for S14 ($p<0.001$); maximal inhibition required concentrations of 10^{-8} mol/l for S28 and higher than 10^{-7} mol/l for S14.

Table 1 summarises the concentrations needed to obtain half-maximal inhibition (ED_{50} values) for these

Table 1. ED_{50} values of somatostatin-induced inhibition of hormone release and cAMP production

		ED_{50} values (mol/l)					
		Inhibition of hormone release			Inhibition of cAMP production		
S14	A cells	10^{-11}	$[4\times10^{-12}; 3\times10^{-11}]$	$p<0.001$	2×10^{-12}	$[10^{-12}; 4\times10^{-12}]$	$p<0.001$
S14	B cells	2×10^{-9}	$[10^{-9}; 4\times10^{-9}]$		2×10^{-9}	$[9\times10^{-10}; 4\times10^{-9}]$	
S28	A cells	2×10^{-11}	$[9\times10^{-12}; 5\times10^{-11}]^a$	NS	8×10^{-12}	$[4\times10^{-12}; 10^{-11}]^a$	NS
S28	B cells	5×10^{-11}	$[3\times10^{-11}; 10^{-10}]^b$		2×10^{-11}	$[10^{-11}; 4\times10^{-11}]^b$	

Data represent mean values with corresponding 95% confidence intervals and were obtained after linear transformation of the sigmoid curves of Figure 3 using a logit approach based on normal distribution and least square regression analysis [28]. Significance of differences between estimated ED_{50} values was assessed with a modified t-test [29]. A cells versus B cells: NS = not significant at $p<0.05$ · S14 versus S28: [a] = not significant at $p<0.05$; [b] = significant at $p<0.001$

effects. For pancreatic A cells, the ED_{50} values of S14 (10^{-11} mol/l) and S28 (2×10^{-11} mol/l) were not significantly different. With pancreatic B cells however, the ED_{50} value of S14 (2×10^{-9} mol/l) was 40-fold higher than that of S28 ($p<0.001$).

Effect of S14 and S28 on cyclic AMP production in pancreatic A and B cells

Pancreatic A cells were suppressed in their noradrenaline-induced cyclic AMP production when exposed to S14 or S28 (Fig. 2, left panel). Inhibition occurred at 10^{-11} mol/l ($p<0.05$) and was complete at 10^{-10} mol/l. At 10^{-11} mol/l, S14 was a more potent suppressor than S28 ($p<0.01$).

S14 and S28 also suppressed cyclic AMP production in pancreatic B cells stimulated by glucagon and glucose (Fig. 2, right panel). Significant inhibition was induced at 10^{-11} mol/l S28 ($p<0.05$) but required 1000-fold higher concentrations of S14. Complete suppression was achieved by 10^{-9} mol/l S28 and 10^{-7} mol/l S14. In the intermediate concentration range, S28 appeared a more powerful inhibitor than S14 (Fig. 2).

The ED_{50} values for the somatostatin inhibition of cAMP production in A cells were 2×10^{-12} mol/l with S14 and 8×10^{-12} mol/l with S28 ($p>0.05$; Table 1). In pancreatic B cells, the ED_{50} value of S14 (2×10^{-9} mol/l) was 100-fold higher than that of S28 ($p<0.001$; Table 1). The ED_{50} values for somatostatin-induced inhibition of hormone release were not significantly different from those for the inhibition of cAMP production in the corresponding conditions (Table 1).

Sensitivity of pancreatic A and B cells for S14 and S28

In order to compare the sensitivities of pancreatic A and B cells for S14 and S28, the respective effects of the peptides were expressed as a percent of the total suppressible activity, be it hormone release (Fig. 3 - left panels) or cyclic AMP production (Fig. 3 - right panels). For somatostatin-14, it was noted that the process of hormone release and that of cAMP production could be suppressed in the picomolar range in the case of A cells

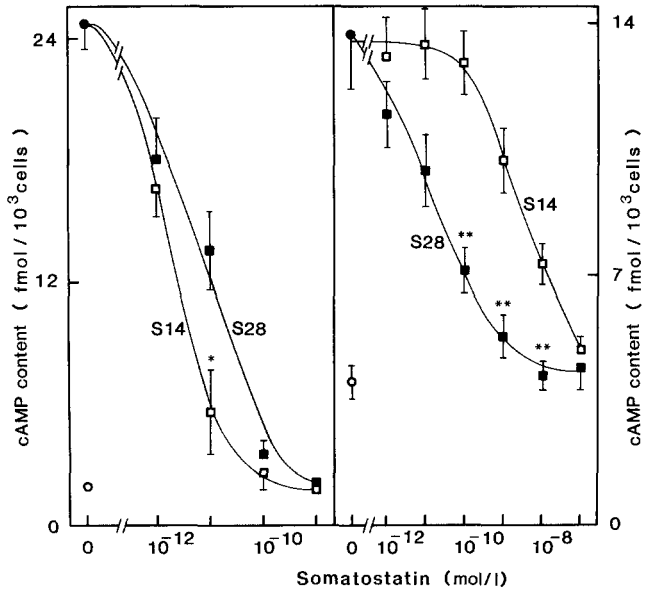

Fig. 2. Effect of S14 and S28 on cyclic AMP levels in purified A and B cells. A cells (left panel) and B cells (right panel) were incubated with different S14 (□) or S28 (■) concentrations (see legend of Fig. 1), and cyclic AMP measured in the cell pellets. ○ basal control (EH with 1.4 mmol/l glucose); ● stimulus control (no somatostatin added). Data represent mean values ± SEM of 5 experiments. The statistical significance of differences between effects of equimolar S14 and S28 concentrations was calculated by the paired Student's t-test. *: $p<0.05$; **: $p<0.001$

but only in the nanomolar range in the case of pancreatic B cells (Fig. 3, top panels). On the contrary, a similar sensitivity of A and B cells for somatostatin-28 was noted as the dose-response curves for this peptide could be superimposed (Fig. 3 lower panels). The ED_{50} values for the inhibitory effects of S14 on hormone release and cAMP production were 200 to 1000-fold higher in pancreatic B cells than in pancreatic A cells ($p<0.001$ - Table 1). On the other hand, no significant difference was found in the ED_{50} values of the S28 effects on both cell types.

Effect of somatostatin-14 analogues

In view of the observed difference in sensitivity of pancreatic A and B cells to somatostatin-14, we examined the cell-selectivity of two synthetic somatostatin-14

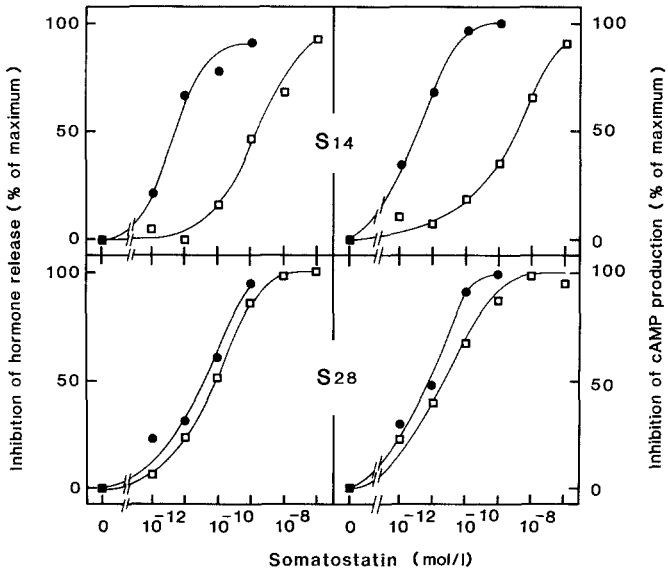

Fig. 3. Comparison of the sensitivity of purified A and B cells to somatostatin inhibition. Data are derived from the experiments shown in Figures 1 and 2. Effects of S14 (upper panels) and S28 (lower panels) on glucagon (●) and insulin (□) release (left panels) and on cyclic AMP production in A (●) and B cells (□) (right panels). Data are expressed as % of maximal inhibition, which was observed at 10^{-7} mol/l somatostatin for B cells and 10^{-9} mol/l for A cells

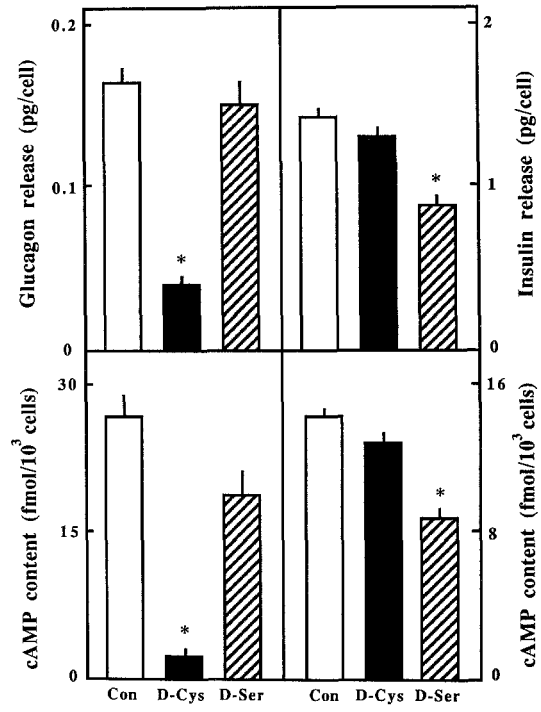

Fig. 4. Effect of S14 analogues on hormone release (upper panels) and cyclic AMP content (lower panels) from A cells (left panels) and B cells (right panels). The cells were incubated for 15 min with either [D-Trp^8, D-Cys^{14}]S14 (D-Cys; 10^{-10} mol/l) or desAsn^5[D-Trp^8, D-Ser^{13}]S14 (D-Ser; 10^{-9} mol/l); no somatostatin was added in the control condition (Con). Data represent mean values ± SEM of 4 experiments. The statistical significance of differences between conditions with somatostatin analogues and control was calculated with the unpaired Student's t-test and was corrected with the Bonferroni method for multiple comparisons [27]. *: $p < 0.01$ versus control

analogues, namely [D-Trp^8, D-Cys^{14}]S14 (D-Cys in Fig. 4) and desAsn^5[D-Trp^8, D-Ser^{13}]S14 (D-Ser in Fig. 4).

At a concentration of 10^{-10} mol/l, [D-Trp^8, D-Cys^{14}]S14 exerted effects that were more pronounced than those observed with S14: the functions of pancreatic A cells were almost completely suppressed, whereas no significant inhibition was measured with pancreatic B cells (Fig. 4). When desAsn^5[D-Trp^8, D-Ser^{13}]S14 was tested - even at a 10-fold higher concentration - it did not suppress glucagon release or cyclic AMP production in the A cell preparations (Fig. 4), but reduced insulin release and cAMP production in pancreatic B cells ($p < 0.01$) similar to equimolar S14 concentrations (Fig. 4).

Discussion

The availability of purified pancreatic A and B cells permits to investigate in further detail the inhibitory action of somatostatin upon the endocrine pancreas [22, 23, 25].

Our results confirm that somatostatin-14 can inhibit glucagon and insulin release via direct interactions with, respectively, A and B cells [22, 23] and extend this property to somatostatin-28. The inhibitory effects of S14 and S28 on hormone release were dose-dependent and closely parallelled by similar reductions in cyclic AMP formation as indicated by the respective ED_{50} values. A reduced adenylcyclase activity may thus mediate - at least in part - the inhibitory effect of somatostatin on pancreatic hormone release [22, 25]. Somatostatin also suppresses cyclic AMP production in other cell types [30-32]. Its ability to inhibit glucagon or insulin release may thus depend on the degree to which these processes are activated by the adenylcyclase unit at the time of exposure. The possibility nevertheless remains that somatostatin is also capable of influencing the hormone release process via additional, cyclic AMP-independent, routes [33, 34].

The pancreatic A cells were inhibited by low somatostatin concentrations (ED_{50} values between 2×10^{-12} mol/l and 2×10^{-11} mol/l). These levels can be considered as physiologic even if pancreatic somatostatin has no direct access to the neighbouring A cells. Plasma somatostatin levels vary between 10^{-12} mol/l and 5×10^{-11} mol/l [35] and may thus influence the rate of glucagon release *in vivo*. Since S14 seems - in this concentration range - a more potent inhibitor than S28, it is conceivable that the relative proportions of both forms will further modulate the degree of inhibition.

Pancreatic B cells were only suppressed by S14 concentrations of 10^{-9} mol/l or higher. This relatively low sensitivity to the exogenous hormone should not be attributed to the presence of endogenously released S14 as the purified B cell preparation contains less than

3 percent D-cells [19] and as the endogenous somatostatin levels measured in the controls were lower than 10^{-11} mol/l [22]. It is also unlikely that it is caused by damage during tissue dissociation since pancreatic A cells - which were exposed to the same procedure as the B cells - exhibited an exquisite responsiveness to S14. Moreover, the isolated B cells have been previously shown to exhibit high affinity binding sites for other peptides [26, 36], and were found, in this paper, to be sensitive to concentrations of S28 in the picomolar range, a sensitivity which is similar to that previously noted in intact pancreata [16, 17]. When extrapolated to the *in vivo* situation, these data suggest that the regulation of insulin release by somatostatin-14 is only conceivable if pancreatic somatostatin reaches the islet B cells before it is diluted in the general circulation. It is still unclear whether such direct access exists via the intercellular space or via the local circulation. According to Bonner-Weir and Orci [37] and Kawai et al. [38], the intra-islet vascularisation does not permit an influence via the local circulation. The fact that purified B cells were nearly 50-fold more sensitive to S28 (Table 1), suggests a regulatory role for circulating S28, which mainly originates from the gastrointestinal tract.

A comparison of the ED_{50} values indicates that S14 is approximately 500-fold more potent in pancreatic A cells than in B cells. This difference in biological activity may result from the existence of two different somatostatin receptors, i.e. high affinity S14 receptors on A cells and low affinity binding sites for S14 on B cells. The results obtained with S14 analogues support this view, as one analogue acted preferentially on A cells - reproducing the marked suppressive effects of S14 - whereas the other remained without effect on A cells but induced a modest inhibition in B cells. The *in vitro* findings with these drugs are in good agreement with previous *in vivo* observations [39-41]. The physiologic meaning of the different sensitivity of pancreatic A and B cells to S14 is still unclear. It may represent another example of the divergent regulation which can be expected for the release of counteracting hormones [24]. The respective hormone responses thus become dependent on the origin and the extent of the increased S14 levels. Locally, this different sensitivity to S14 may be part of a functional integration of the various islet cell types. On the other hand, the ED_{50} values for the suppressive effects of S28 indicate that both cell types are highly and equally sensitive to this peptide. Pancreatic A and B cells seem therefore to be equipped with high affinity S28 receptors.

The similarity in S28 sensitivity and the difference in S14 sensitivity suggest that the putative somatostatin receptor on A cells is to be distinguished from that on B cells. Cell-selective binding of S14 and S28 has also been suggested on the basis of autoradiographic studies in unpurified rat islet cell monolayers [42]. Binding studies with intact purified A and B cells are of course necessary to further document the existence of different somatostatin receptors on both cell types. These experiments will also tell whether the high affinity S28 receptors on B cells provide low affinity binding sites for S14.

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