Kinetics of Somatostatin Receptor Migration in Isolated Pancreatic Islets

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SUMMARY

Eighty-seven percent of the total cellular pool of somatostatin (SRIF) receptors in pancreatic islets are located intracellularly. Upon glucose stimulation (300 mg/dl) of insulin release, 8–15% of intracellular SRIF receptors are translocated to the plasma membrane. Affinity of SRIF receptors does not change during their migration and the total cellular pool of receptors remains constant. With prolonged glucose stimulation, surface membrane somatostatin receptor concentration reaches a maximum level at 60 min. DIABETES *31*: 467–469, May 1982.

Intracellular receptors for polypeptide hormones have been recently described. The role of secretion vesicles in intracellular receptor transport was suggested[1–3] and confirmed by finding specific somatostatin (SRIF) receptors in the secretion vesicles from both the pituitary and pancreatic islets.[4–6] The critical event in the modulation of SRIF surface receptor concentration appears to be the migration of secretion vesicles to the plasma membrane during emiocytosis.[5,6] This process promotes increased transport of the receptors from the Golgi apparatus to the plasma membrane and confers upon the plasma membrane a receptor for SRIF.

The purpose of this work was to assess: (1) how large is the total cellular pool of SRIF receptors, (2) what portion of the total cellular pool of receptors is located on the cell surface, and (3) what portion of the intracellular SRIF receptors is translocated to the cell surface with enhanced insulin release.

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MATERIALS AND METHODS

Male Sprague-Dawley rats (250–350 g) were used in this study. Pancreatic islets were isolated using the collagenase digestion method as described previously.[4,5] While one group of islets was left intact, the others were homogenized prior to binding experiments. Both intact and homogenized islets were incubated with either 30 or 300 mg/dl glucose for 15, 30, 60, and 120 min at 37°C. ^{125}I-SRIF binding was initiated at the end of these incubations and was carried out at 4°C for 16 h as previously described.[5] In experiments with intact islets, SRIF binds to its surface receptors, whereas being incubated with homogenized islets it binds to the total cellular pool of receptor. All results were corrected for nonspecific binding and represent the mean values of 4 independent experiments conducted in either duplicate or triplicate.

RESULTS

Figure 1A demonstrates that SRIF binding to the total cellular pool of receptors is much greater than SRIF binding to the surface of the pancreatic islets incubated with either 30 or 300 mg/dl glucose. These experiments confirm the fact that the major pool of SRIF receptors is located intracellularly. Scatchard analysis of SRIF binding (Figure 1B) to the receptors located on the surface of pancreatic islets and to the total pool of cellular SRIF receptors reveals that the total cellular pool of SRIF receptors is about eight times greater than the number of its surface receptors. The affinity of all SRIF receptors is identical regardless of where the receptors are located. We then calculated the percent of the total cellular pool of SRIF receptors present on the surface of the pancreatic islets. When islets were kept in a basal, low glucose concentration medium (30 mg/dl), SRIF binding did not change significantly throughout the experimental period. The number of SRIF receptors remained steady and varied between 8 and 12% of the total cellular receptor pool (Figure 2A). When the islets were placed into a high glucose concentration medium (300 mg/dl), the number of SRIF receptors discovered on the surface of the pancreatic islets vary rapidly over the first 15 min, reached a maximum (24%

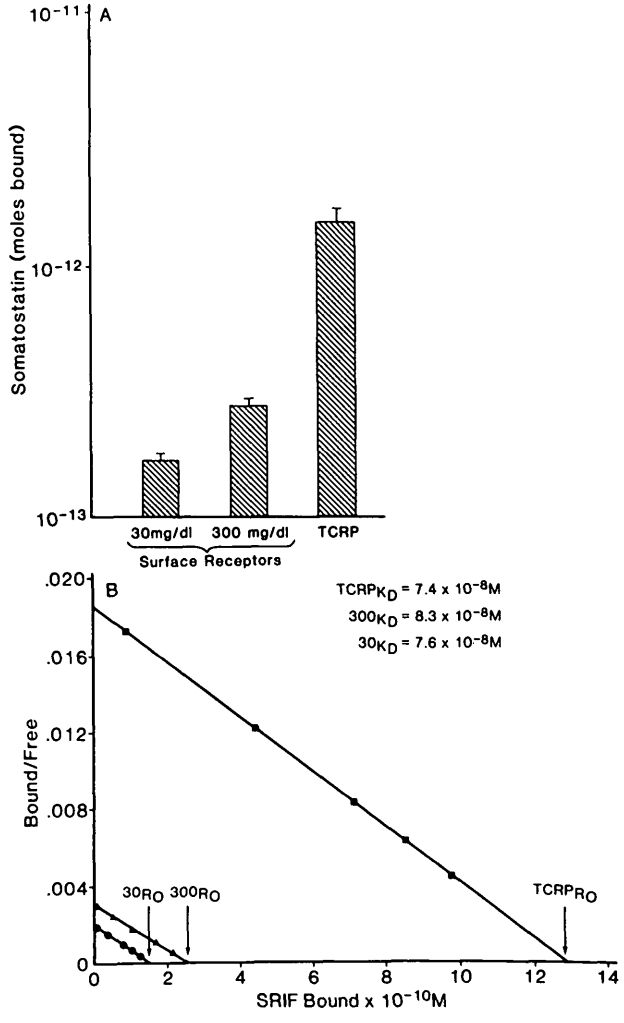

FIGURE 1. (A) Specific SRIF binding to the intact (surface receptors) and homogenized (total cellular receptor pool-TCRP) pancreatic islets. Isolated islets were incubated in media containing 30 or 300 mg/dl glucose for 30 min at 37°C. A second group of islets was homogenized to yield the total cellular receptor pool. Binding assessed by incubation with ^{125}I-SRIF at 4°C for 16 h. (B) Scatchard analysis of binding experiments. Intact and homogenized pancreatic islets were incubated with ^{125}I-SRIF in the presence of increasing concentrations of unlabeled SRIF. The amount of bound SRIF (B) is plotted against bound/free ratio (B/F). Ro indicates the number of SRIF receptors.

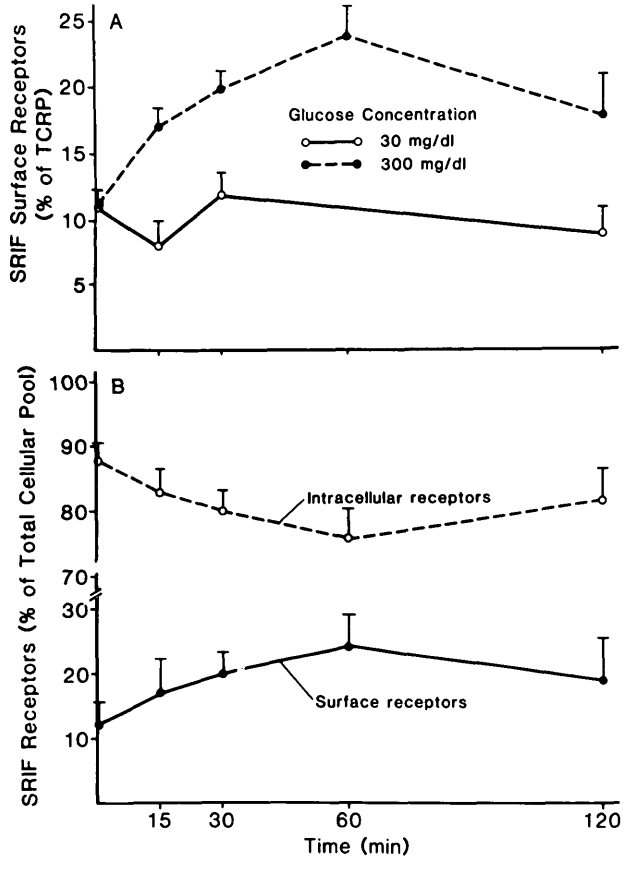

FIGURE 2. (A) SRIF surface receptors in pancreatic islets. Intact isolated islets were incubated in either 30 or 300 mg/dl glucose. At the indicated periods of time, the SRIF binding reaction was initiated in intact islets and islets subjected to homogenization (TCRP). (B) ^{125}I-SRIF binding to both intact and homogenized islets incubated with 300 mg/dl glucose. Intracellular receptor concentration is represented by the difference between total cellular receptor pool (homogenized islets) and surface receptors (intact islets).

of the total cellular receptor pool) at 1 h and slightly declined at 2 h of incubation. The number of the total cellular pool of receptors remained constant throughout the experimental period of time. We calculated and compared the kinetics of the SRIF receptors on the surface of the islets and those located intracellularly (Figure 2B). An inverse relationship exists between SRIF receptors: an increase in the number of surface receptor corresponds to a fall in the number of intracellular receptors. Interestingly, when intact islets incubated in high glucose concentration were returned into low glucose concentration media, the number of surface SRIF receptors remained elevated (20% of TCRP) for 2 h, indicating a relatively slow rate of receptor turnover.

DISCUSSION

Intracellular somatostatin receptors were first described by Ogawa et al. in 1977.[7] We have recently observed the link between secretion vesicle migration and somatostatin binding in isolated pancreatic islets.[5] The role of Golgi vesicles in intracellular transport of receptors has been suggested previously by several investigators.[1,2] Although the existence of intracellular receptors for a variety of hormones has been demonstrated,[1,2,8–12] it was only recently shown that specific SRIF receptors are present in secretion vesicles isolated from both pituitary and pancreatic islets.[5,6] Our present findings not only confirm the fact of the existence of intracellular SRIF receptors, but demonstrate that the total cellular pool of receptors is about 8 times greater than the number of its surface receptors. The affinity of intracellular SRIF receptors is identical to that of cell surface receptors. The similarity between intracellular and cell surface receptors has been observed by others as well.[1,9,11,12]

When islets were incubated with high concentration of glucose, rapid translocation of intracellular receptors to the cell surface was observed. The number of SRIF surface receptors rose from 12 to 24% of the total cellular pool of receptors at 1 h of incubation. This figure is compatible with the previously observed 180–190% increase in glucose-induced insulin release.[5]

In view of the previous observations concerning the presence of somatostatin binding sites on isolated secretion vesicles (both from the anterior pituitary gland and pancreatic islets),[5] it was not unexpected that islet homogenates

TABLE 1
Total cellular pool of somatostatin receptors—^{125}I-SRIF binding to homogenized pancreatic islets*

Pancreatic islets	Glucose (mg/dl)	Time of incubation (min) 30	60	120
Incubated with glucose before homogenization	30	19.7%	16.6%	19.2%
	300	20.7%	17.3%	18.1%
Homogenized and then incubated with glucose	30	19.5%	18.8%	19.6%
	300	19.3%	18.1%	19.1%

* Results are expressed as mean values of four experiments.

showed a considerable number of binding sites. We had entertained the possibility that increased SRIF binding to homogenized islets may be due to an unmasking of existing SRIF plasma membrane receptors. However, solubilization of pituitary plasma membranes and secretion vesicles in either SDS or Triton-X did not alter the magnitude of SRIF binding (data not shown). Nevertheless, the possibility of unmasking islet surface receptors by homogenization cannot be discounted entirely. It would be more likely that if a conformational change was induced in membrane receptors due to homogenization, that this would have been reflected in some alteration in receptor affinity and this was not observed.

In like manner, the question can be legitimately posed as to whether the increase in surface membrane receptor concentration with glucose might not be a direct consequence of glycosylation of receptors. This appears unlikely since the total cellular receptor pool (i.e., binding of SRIF to homogenized islets) was not affected by the presence of high or low glucose.

The total cellular receptor pool remained unchanged by the sequence followed in the experimental protocol. Islets homogenized prior to the incubation with high or low glucose concentration bound the same amount of SRIF as did islets incubated with glucose first and then subsequently homogenized (Table 1). In view of these observations, it seems unlikely that proteases released by homogenized islets alter SRIF binding. We believe that these results represent the actual total cellular pool of SRIF receptors.

In contrast to rapid mobilization of intracellular SRIF receptors with glucose stimulation, the rate of disappearance of these receptors from the cell surface when the islets were returned into low glucose concentration media was relatively slow, suggesting a slow rate of SRIF receptor turnover.

In summary, these findings led us to believe that there exists a major pool (87%) of somatostatin receptors in the intracellular compartment. Glucose rapidly initiates somatostatin receptor translocation to the plasma membrane. However, only 8–15% of the intracellular somatostatin receptor pool is translocated to the plasma membrane with stimulation of insulin release. These numbers must be considered relative, because somatostatin released from the islets during homogenization may compete with ^{125}I-SRIF for binding sites. Nevertheless, the number of intracellular SRIF receptors translocated to the plasma membrane is consistent with the amount of insulin released from the intracellular stores (5–10%) following glucose stimulation.[13]

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