Role of Insulin Secretagogues in the Regulation of Somatostatin Binding by Isolated Rat Islets

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A B S T R A C T   To study the possible role of the secretion vesicle in ligand-receptor interaction, somatostatin binding was measured in islets in the presence of various substances known to promote secretion vesicle migration and fusion with the plasma membrane and insulin release. Rat islets were incubated with glucose, 30 and 300 mg/dl, for 60 min. After incubation, somatostatin binding was measured. In islets preincubated with glucose, 300 mg/dl, somatostatin binding was increased 250% when compared with glucose, 30 mg/dl ($P < 0.001$). Concomitant with enhanced somatostatin binding, insulin secretion was increased. Galactose, 300 mg/dl, did not stimulate insulin release, and somatostatin binding was unchanged from control levels. The increase in somatostatin binding with glucose was accounted for by a 186% increase in receptor concentration with no change in receptor affinity. Tolbutamide increased somatostatin binding by more than twofold, accompanied by a similar increase in insulin release. Secretion vesicles isolated from the islet exhibited somatostatin binding. We conclude that, first, somatostatin binding is increased concomitantly with the migration and fusion of the secretion vesicle with the plasma membrane and/or the release of insulin; second, enhanced somatostatin binding occurs as a consequence of an increased receptor concentration; and third, augmented somatostatin binding occurring with hormone release may provide a critical constraint in the regulation of secretory events.

INTRODUCTION

In cell systems capable of secreting polypeptide hormones, the secretion vesicle may play a multifaceted

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role. The primary function of the secretion vesicle is to act as a reservoir for storage of hormone and to facilitate intracellular transport of hormones (1, 2). Secretory products are probably undergoing some processing within the vesicle (1, 3). The secretion vesicle itself may also exert some role relative to the regulation of hormone secretion. This is suggested by the observations that the secretion vesicles isolated from pancreatic islets and the anterior pituitary gland possess cyclic AMP-dependent protein kinase activity (4, 5). Furthermore, it has been demonstrated that somatostatin specifically inhibits this protein kinase (6).

Secretion vesicles isolated from the anterior pituitary gland were found to bind somatostatin with an affinity exceeding that observed in plasma membranes, and were fivefold richer in somatostatin binding sites (7). We have proposed the thesis that during the process of emiocytosis, the secretion vesicle may bring to the plasma membrane an effector unit (i.e., cyclic AMP-dependent protein kinase), as well as a high-affinity binding site for somatostatin (7).

If this thesis is correct, one would anticipate that during the process of emiocytosis and hormone release, cellular binding of somatostatin would be enhanced. The purpose of the present study is to measure somatostatin binding in isolated rat islets under the influence of various secretagogues that are known to promote secretion vesicle migration and insulin release.

METHODS

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[1] *Abbreviations used in this paper:* SRIF, cyclic somatostatin; TRH, thyrotropin-releasing hormone.

School, Rochester, Minn. Sephadex G-25 and dextran T70 were purchased from Pharmacia Fine Chemicals, Inc., Piscataway, N. J.

Isolation of islets and secretion vesicles. Male Sprague-Dawley rats weighing 300–450 g were obtained from the Simonson Laboratories, Gilroy, Calif. The animals were allowed food and water *ad lib.* Anesthesia was induced with pentobarbital (Diabutal), 45 mg/kg body wt. Pancreatic islets were isolated by the method of Lacy and Kostianovsky (8) and subjected to the Ficoll gradient separation of Shibata et al. (9). The isolated islets were collected using a micropipette and preincubated for 30 min at 37°C in Krebs-Ringer bicarbonate buffer containing 30 mg/dl glucose and 1 mg/ml bovine serum albumin (BSA). The buffer had been previously gassed with 95% O_2/5% CO_2 for 45 min and adjusted to pH 7.4. Islet secretion vesicles were isolated according to the method of Leitner et al. (10).

Iodination procedure. Tyr[1]-somatostatin was labeled with ^{125}I according to the method of Harris et al. (11). ^{125}I-Tyr[1]-somatostatin was eluted from a Sephadex G-25 (fine) column (1.5 × 60 cm) with 0.1 M acetic acid containing 0.1% gelatin. The peak immunoreactive labeled product, as determined by the method of Gerich et al. (12), had a specific activity >140 Ci/mmol.

Incubation protocol for insulin release and somatostatin binding studies. Following preincubation, 10 islets were transferred into a series of 12 × 75-mm glass tubes containing 200 μl of the gassed Krebs-Ringer buffer with 30 mg/dl glucose and 1 mg/ml BSA at 2°C. This was followed by the addition of 800 μl of gassed Krebs-Ringer bicarbonate buffer containing 1 mg/ml BSA and sufficient glucose and/or tolbutamide to yield final concentrations of 300 mg/dl glucose, 30 mg/dl glucose, and 30 mg/dl glucose plus 400 μg/ml tolbutamide. In those experiments designed to study the effect of galactose on somatostatin binding and insulin release, similar buffers were prepared with galactose being substituted for glucose. The sample tubes were then incubated at 37°C for 1 h, cooled to 2°C for 5 min and centrifuged at 800 g for 5 min at 4°C. An 800-μl aliquot of the medium was carefully removed from each tube for determination of insulin release by double antibody radioimmunoassay. The islets in the remaining media were saved for somatostatin binding studies (referred to as incubated islets).

The technique for determining somatostatin binding described elsewhere has been modified and applied to intact islets and isolated secretory vesicles (7, 13, 14). A buffered medium (900 μl) consisting of 25 mM EDTA, 0.1% BSA, and 500 KIU/ml Trasylol in 50 mM Tris, pH 8.0, was added to the 200 μl of medium containing the incubated islets. Finally, 15 μl of label containing 2 ng ^{125}I-SRIF (250,000 cpm) was introduced into the incubation medium. In the studies directed at determining displacement of labeled SRIF, 900 μl of buffered medium containing unlabeled SRIF was added to yield final concentrations of 0, 0.01, 0.025, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, or 20 μg/ml. In all studies, samples were prepared in triplicate. Three "total count" tubes were included in each incubation. All preparatory steps were carried out at 2°C, and binding was determined by incubation of the samples at 4°C for 18 h.

The binding reactions were terminated by centrifuging the samples at 800 g for 5 min at 4°C. A 900-μl aliquot of the medium was removed and discarded (without disturbing the islet pellet). Ice-cold ethanol was added to the islets and remaining medium to yield a final concentration of 85% (vol/vol). The samples were allowed to stand at 2°C for a period of 10 min and then centrifuged at 1,800 g for 10 min at 4°C. The entire supernate was carefully removed and discarded. Pellet radioactivity was determined in a Searle Analytic 1185 automatic gamma counting system (Searle Radiographics, Inc., Des Plaines, Ill.).

Specific binding was determined as the difference between the samples containing 0 and 20 μg/ml unlabeled SRIF, these representing "total binding" and "nonspecific binding," respectively. Nonspecific binding comprised 28±4% of total binding at the high glucose concentrations and 54±6% of total binding at low glucose levels. The absolute magnitude of nonspecific binding was not influenced by any of the treatments employed in these experiments and represented 0.36±0.05% of the total available radioactivity.

Dissociation constants were calculated by least squares regression analysis, and where appropriate, statistical analyses were performed utilizing Student's *t* test for unpaired data.

Finally, studies were directed at determining possible somatostatin binding by isolated islet secretion vesicles. Secretory vesicles were obtained from 2,000 islets. This fraction contained 5.8% of the total protein and 42.8% of the total insulin content. Thus, the secretion vesicle fraction represented an 18-fold enrichment in insulin content (microunits per microgram protein) when compared with homogenate. The purity of this fraction has been established in previous publications (10, 15).

The vesicles were suspended in 50 mM Tris, pH 8.0. Total and nonspecific somatostatin binding was determined by adding 100 μl of appropriate buffer reagent to a 100-μl aliquot of the secretory vesicle fraction containing 10 μg protein. Introduction of label, incubation, and termination of the binding reaction were as before.

RESULTS

Fig. 1 demonstrates that when isolated rat islets were incubated with 300 mg/dl glucose as compared with 30 mg/dl glucose, somatostatin binding was increased
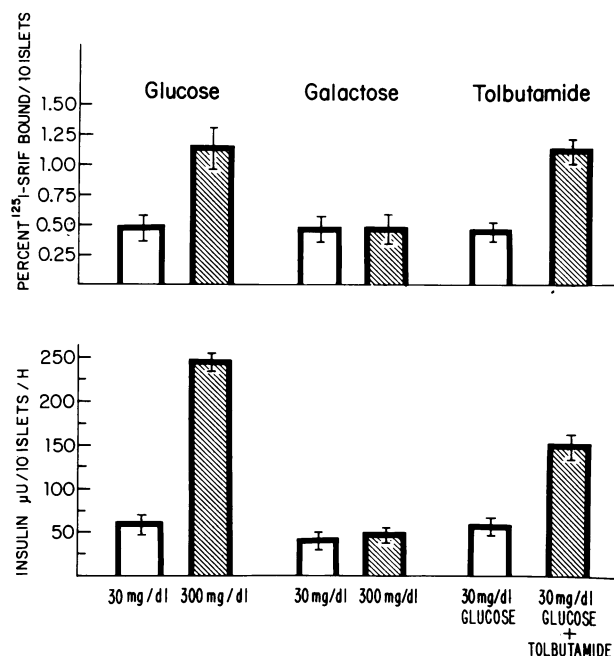

FIGURE 1 Somatostatin binding (upper bar graph) and insulin release (lower bar graph) in isolated pancreatic islets incubated with glucose (*n* = 25), galactose (*n* = 10), or tolbutamide (*n* = 8). Shown is the percent of somatostatin bound per 10 islets corrected for nonspecific binding. Values are mean±SEM.

249±35% ($P < 0.001$). Concomitantly with the increases in somatostatin binding, insulin levels increased from 58±4 to 250±8 μU/ml ($P < 0.001$). In contrast, incubation in similar concentrations of galactose produced no similar enhancement of somatostatin binding. No increase in insulin secretion was observed with galactose, which is consistent with previous observations in this regard (16, 17). Tolbutamide (400 μg/ml) increased somatostatin binding by 265 ±14% ($P < 0.001$). In this setting, insulin levels increased from 53±8 to 151±16 μU/ml ($P < 0.001$).

Islets were incubated at insulin concentrations that reflected the levels of insulin release obtained at a glucose level of 300 mg/dl. Under these circumstances somatostatin binding was not increased. Thus, the effect of glucose in enhancing somatostatin binding could not be attributed to increased media insulin levels.

Fig. 2 demonstrates the displacement of labeled somatostatin by the addition of unlabeled somatostatin. The displacement curves show somatostatin binding in islets that have been incubated at both high and low glucose concentration. At all levels of added somatostatin, those islets incubated in a glucose concentration of 300 mg/dl showed greater binding than those islets incubated at the lower glucose concentration. In conformity with previous data, there was no displacement of labeled somatostatin by two low molecular weight peptides, thyrotropin-releasing hormone (TRH) and luteinizing hormone releasing factor (13).

The displacement data analyzed according to the method of Scatchard (18) are shown in Fig. 3. It is apparent that the increase in somatostatin binding in those islets incubated in the high glucose concentration is not accompanied by a change in binding affinity. In contrast, the high-affinity receptor concentration (Ro) has increased from 1.53±0.28 to 2.58±0.35 × 10^{-10} M in the islets incubated in glucose (300 mg/dl) as compared with the low glucose level ($P < 0.001$). These values have been corrected for the contribution of low-affinity binding. Whether the curvilinearity of the Scatchard plot represents negative cooperation or two classes of binding sites cannot be answered with the present data. Because the slopes of the low-affinity components are not significantly different, it is apparent that if one analyzes either the high-affinity components or the total curves (average affinity), the change in islet somatostatin binding with high glucose concentration is due to an increase in receptor concentration.

Previous studies relative to somatostatin binding in secretion vesicles were performed using bovine anterior pituitary glands (13). Because of the possible role of the secretion vesicle in modulating somatostatin binding, it was critical to establish that such binding exists in the secretion vesicle fraction isolated from rat islets. Total somatostatin binding by islet secretion vesicles was 2.11±0.3%, of which 26.1±1.1% was nonspecific binding. Specific binding by the secretion vesicle was 1.71±0.39 × 10^{-14} mol/μg protein compared with 4.83±0.80 × 10^{-15} mol/μg protein for the intact islet.
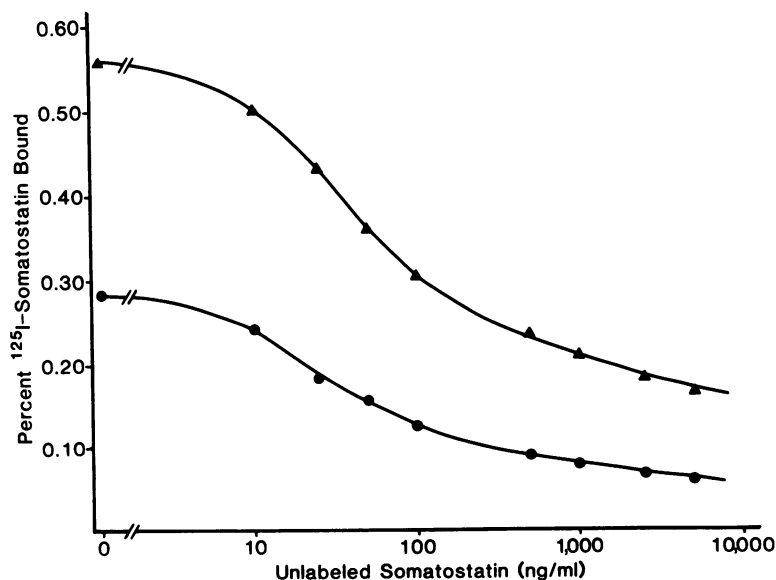

FIGURE 2 Competition curves for somatostatin. Displacement of labeled somatostatin by unlabeled somatostatin. Shown are mean values (n = 8) for binding obtained with islets incubated at glucose concentrations of 300 mg/dl (▲) and 30 mg/dl (●).
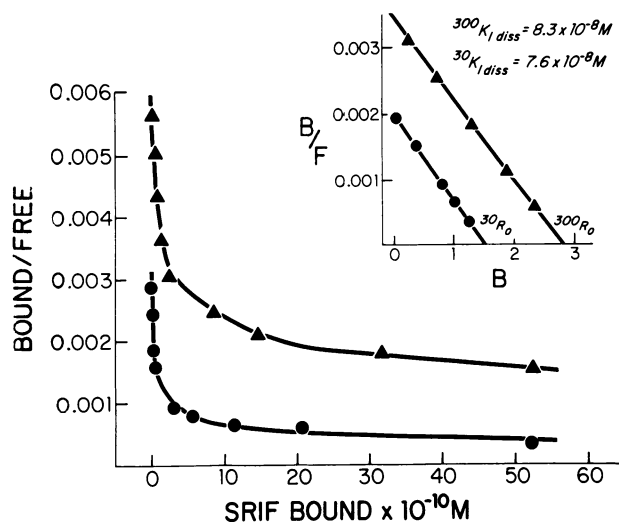
FIGURE 3 Scatchard analysis of somatostatin displacement data. Inset is a Scatchard plot for the high-affinity component corrected for the contribution of the low-affinity binding sites. Shown are the dissociation constants and the ×-intercepts (Ro) for 30 and 300 mg/dl glucose.

DISCUSSION

The present data suggest that with insulin secretion and/or the event of the migration and union of the secretion vesicle with the plasma membrane, somatostatin binding is enhanced. The data indicate that the enhanced binding of somatostatin associated with various insulin secretagogues occurs as a result of an increase in receptor concentrations rather than a change in binding affinity. Schonbrunn and Tashjian (19) reported increased binding of somatostatin in the presence of TRH in cultured pituitary tumor cells. As in the present study, the enhanced binding of somatostatin was related to an increase in the concentration of receptor sites with no change in affinity. These investigators postulated that TRH may exert its effect on the internalization or processing steps for the somatostatin receptor. Little is known about the kinetics of receptor degradation. Whether the various secretagogues may have altered the rates of internalization and/or degradation of the somatostatin receptor has not been investigated.

Previous data from this laboratory using subcellular organelles isolated from bovine anterior pituitary glands suggested that there exists a specific somatostatin binding site in the secretion vesicle (7). It was postulated that perhaps during the process of emiocytosis, the secretion vesicle confers upon the plasma membrane a high-affinity binding site for somatostatin. The present set of experiments performed in pancreatic islets is consistent with this thesis insofar as somatostatin binding is enhanced with various secretagogues, this enhancement being due to an increase in receptor

concentration. This thesis is further supported by the fact that the islet secretion vesicles exhibited somatostatin binding activity that exceeds that found in the intact islet.

An alternate hypothesis is that, as a result of some change in the plasma membrane consequent to the event of hormone release, preexistent binding sites are unmasked, which allows an increased amount of somatostatin to be bound. This latter mechanism may involve the transfer of binding sites from the secretion vesicle to the plasma membrane during the process in which these two subcellular organelles become united. The possibility exists that somatostatin receptors are synthesized within the plasma membrane, and that the secretion vesicle exerts no function in modulating somatostatin binding. In the studies of Schonbrunn and Tashjian (19), the TRH-induced increase in somatostatin receptor number was not prevented when protein synthesis was inhibited by cycloheximide.

In any event, the net effect would be the same under each hypothesis; that is, as a result of various secretagogues promoting enhanced hormone release, the plasma membrane would undergo an alteration in which somatostatin binding is increased, thereby providing a possible constraint in modulating further glucose and tolbutamide-induced insulin release. However, the islets are a heterogeneous tissue, containing various cells that are known to be effected by somatostatin. Beta cells represent 75–80% of islet mass. Nevertheless, with glucose increasing somatostatin binding, it remains unanswered whether this phenomenon is also occurring in the glucagon-secreting alpha cell. Thus, the interrelationships may be quite complex within somatostatin binding and action.

With the present evidence, it is appropriate to conceive of the secretion vesicle as playing a critical role in modulating hormone release, rather than simply acting as a storage unit and as an intracellular hormone transport system. The secretion vesicle may play a dual role, possessing both the effector unit (i.e., cyclic AMP-stimulated protein kinase) and the high-affinity binding receptor for somatostatin. The regulation of secretory events may be related to biochemical or molecular alterations occurring within the secretion cells at the junction of the secretion vesicle with the plasma membrane. In the case of somatostatin, this hormone would inhibit cyclic AMP-stimulated protein kinase activity at a specific locus in the cell (e.g., the junction of the secretion vesicle with the plasma membrane), rather than affecting a number of cellular processes which may be controlled by cyclic AMP-stimulated protein kinase.

While such a formulation may explain how the secretion vesicle may specifically control the events of hormone secretion, it does not explain the diverse actions of somatostatin in other tissues in which no

secretion vesicles are present (e.g., the gastrointestinal tract). Nevertheless, in those tissues secreting hormones by a mechanism involving granule extrusion, this model of somatostatin binding and action and the role of the secretion vesicle therein is consistent with available data.

ACKNOWLEDGMENTS

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