Differential control of insulin secretion and somatostatin-receptor recruitment in isolated pancreatic islets

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Somatostatin receptors appear to be localized to secretory granules in pancreatic islet homogenates. Recruitment of these receptors to the islet-cell surfaces may mark the contact event between secretory granules and plasma membranes before release of insulin by fission. Isethionate, an impermeant anionic replacement for chloride, blocks the release step but does not affect receptor recruitment. By contrast, low concentrations of phenothiazine drugs, such as trifluoperazine and promethazine, inhibit both receptor recruitment and secretion. Scatchard analysis of phenothiazine effects on somatostatin receptors reveals that these drugs reduce the number of receptors but do not affect the affinity of the receptor for somatostatin. These data indicate that membrane contact and fission steps during exocytosis can be biochemically separated.

Hormone secretion by exocytosis involves initial contact between the secretory vesicle and the plasma membrane, and subsequent fission or breakage of the combined membranes to release vesicle contents (Palade, 1975; Pollard et al., 1979, 1981). In the case of insulin secretion from pancreatic β-cells, we found that the major proportion of somatostatin-receptor activity was in the secretory-vesicle fraction of islet homogenates, possibly associated with the β-granules (Leitner et al., 1980). We further suggested that the secretion vesicle may provide the vehicle for translocating the receptor for somatostatin from the Golgi cisternae to the plasma membrane (Mehler et al., 1980; Sussman et al., 1982). This has led us to the proposal that the initial contact event between secretory vesicles and plasma membranes might be investigated by monitoring the recruitment of somatostatin receptors to the cell surface after stimulation by glucose.

As a part of this investigation, we have attempted to control receptor recruitment and insulin differentially by using isethionate, an impermeant anion, as well as certain phenothiazine drugs, including TFP and PMTHZ. Since isethionate may block only the fission step in exocytosis (Pollard et al., 1981), we anticipated that somatostatin-receptor recruitment might be unaffected by this drug. By contrast, the phenothiazines block a variety of Ca²⁺-binding proteins (Pollard et al., 1981) implicated in regulating the initial contact event, leading us to expect that both somatostatin-receptor recruitment and hormone release would be inhibited.

In the present paper, we report that isethionate blocks glucose-induced insulin release but not somatostatin-receptor recruitment. In addition, low concentrations of either TFP or PMTHZ block both receptor recruitment and hormone secretion under the same conditions. We thus conclude that the membrane contact and fission steps of insulin secretion appear to be biochemically separable processes, accessible to analysis by using specific drugs directed against the two steps.

Materials and methods

Pancreatic islets

Pancreatic islets were prepared by the method of Lacy & Kostianovsky (1967) as modified by Mehler
et al. (1980) either from male Sprague-Dawley rats weighing 250–350 g or from the desert sand rat Psammomys obesus. The desert sand rats were obtained from an inbred colony located at the Hadassah Hospital in Jerusalem. After isolation, the islets were kept in a Krebs-Ringer bicarbonate buffer containing 30 mg of glucose/dl and 0.5 mg of bovine serum albumin/ml. The buffer had been gassed with O₂/CO₂ (19:1) for 45 min before use and the pH was 7.4.

Insulin secretion

Ten islets were placed in glass tubes (12 mm x 75 mm) containing 200 μl of buffer, as above at 2°C. To initiate secretion, 800 μl of buffer containing 300 mg of glucose/dl was added and the suspension was incubated at 37°C for 30 min. The reaction was terminated by reducing the temperature to 2°C for 5 min and then centrifuging at 800 g for 5 min at 4°C. Insulin was assayed in an 800 μl portion of the supernatant solution by a modification of the double-antibody radioimmunoassay technique (Morgan & Lazarow, 1963; Mehler et al., 1980). The remaining islet pellet was washed before assay of available somatostatin receptors with 11 vol. of 50 mM-Tris, pH 8.0, containing 0.5 mg of bovine serum albumin/ml. The somatostatin receptor assay was performed as described previously (Mehler et al., 1980; Sussman et al., 1982). All results were corrected for non-specific binding (binding in the presence of 10 μg of unlabelled somatostatin/ml), which was 0.4 ± 0.05% of the total available radioactivity.

In the experiments employing TFP and PMTHZ, the agents are present in the initial 200 μl of media to yield concentrations of 0, 1, 10, 50 or 100 μM. The samples were pre-incubated at 37°C for 15 min. After this pre-incubation, an 800 μl portion of appropriately buffered medium was added to maintain the desired glucose and drug concentrations. The samples were then incubated for an additional 15 min at 37°C. Samples for insulin assay and somatostatin binding were prepared as described above.

In those studies employing sodium isethionate (Sigma), NaCl was replaced in the Krebs-Ringer bicarbonate buffer medium on an equimolar basis to yield the desired final concentrations of the agent.

Chemicals

TFP was donated by the Smith, Kline and French Laboratories, Philadelphia, PA, U.S.A. PMTHZ was obtained from Wyeth Laboratories, Philadelphia, PA, U.S.A. Sodium isethionate was obtained from Sigma Chemical Company, St. Louis, MO, U.S.A. All other chemicals were of reagent grade.

Results

Receptor recruitment and insulin secretion in isethionate medium

Replacement of chloride in the incubation medium by the impermeant anion isethionate inhibits secretion of adrenaline (Pollard et al., 1979, 1981), lysosomal enzymes (Weissman et al., 1978), parathyrin (Brown et al., 1978) and insulin (Somers et al., 1980; Orci & Malaisse, 1980). In the present study, we also found that increasing isethionate levels progressively and completely blocked insulin secretion from isolated pancreatic islets. Indeed, 50% inhibition occurred where isethionate and chloride were in an approximately 1:1 molar ratio (see Fig. 1) (P < 0.001 versus initial insulin release at 120 mM NaCl).

However, as also shown in Fig. 1, glucose-induced somatostatin-receptor recruitment (P < 0.01 for 30 versus 300 mg/dl) was virtually unaffected by progressive replacement of NaCl by sodium isethionate. This marked the first instance in which somatostatin-receptor recruitment had been dissociated from insulin secretion in isolated islets. We tested the

![Fig. 1. Influence of replacement of extracellular chloride by isethionate on glucose-induced insulin secretion and somatostatin-receptor recruitment](image-url)
Insulin secretion and somatostatin-receptor recruitment

In the generality of this result with islets from Sprague–Dawley rats, and essentially identical results were obtained (results not shown).

The isethionate effect on secretion has been previously interpreted in terms of interference with the osmotic process of fission of secretory granules complexed to the plasma membrane (Pollard et al., 1979, 1981). Seen from this viewpoint, the present data might indicate that the initial membrane contact event can be observed in the absence of the ultimate fission process.

Phenothiazine drug effects on somatostatin-receptor recruitment and insulin secretion

Certain phenothiazine drugs block insulin secretion (Gagliardino et al., 1980; Valverde et al., 1981; Janjic et al., 1981) and inhibit the action of specific Ca²⁺-binding proteins such as synexin (Pollard et al., 1981) and calmodulin (Levin & Weiss, 1976). Synexin is sensitive to low concentrations of both TFP and PMTHZ, whereas calmodulin is more sensitive to TFP than to PMTHZ by a factor of at least 100 (Pazoles et al., 1980).

In our present study, we therefore investigated the relative abilities of both TFP and PMTHZ to affect both insulin secretion and somatostatin-receptor recruitment. Fig. 2(a) shows that TFP blocked somatostatin-receptor recruitment (P<0.01) and insulin release (P<0.01) coincidently, with an ID₅₀ of 1µM. PMTHZ (Fig. 2b) was also found to inhibit both receptor recruitment (P<0.01) and insulin release (P<0.01) coincidently, but at a slightly lower ID₅₀ than that of TFP. The two drugs were statistically indistinguishable, however. In either case, loss of receptor recruitment and insulin secretion were highly correlated (r = 0.93, as shown in Fig. 3).

To be certain about the generality of the effect, we again performed these experiments on pancreatic islets from Sprague–Dawley rats. Similar results were obtained, but the absolute potencies of TFP and PMTHZ were somewhat reduced. Thus the ID₅₀ for TFP was 14µM, whereas that for PMTHZ was 5µM.

Influence of phenothiazines on the binding characteristics of somatostatin receptors

To confirm that phenothiazines were inhibiting receptor recruitment and not altering receptor affinity, we measured the number and affinity of islet receptors using Scatchard analysis of competitive binding data. As shown in Fig. 4(a), incubation of islets with either 30 or 300mg of glucose/dl resulted in changes in the number of somatostatin receptors (R₀), with no change in the affinity of the receptors. Similarly, incubation of islets in 300mg of glucose/dl

in the presence of either 10µM-TFP or -PMTHZ resulted only in a reduction in the number of available receptors, without changes in receptor affinity (Fig. 4b). However, at much higher dose levels, the phenothiazines were found to displace somatostatin from the islet surface-membrane receptor, with changes becoming detectable at 50µM (TFP) and at 75µM (PMTHZ). These levels were substantially above the concentration ranges affecting receptor recruitment and insulin secretion in islets of both Sprague–Dawley rats and desert sand rats.
Correlation between recruitment receptor C' and insulin release

Fig. 3. Correlation between inhibition of somatostatin-receptor recruitment and insulin secretion in different concentrations of either TFP (●) or PMTHZ (○). Results for both insulin release and somatostatin-receptor recruitment represent the change in the difference between results obtained for buffer containing 300mg and 30mg of glucose/dl. Abbreviation used: SRIF, somatostatin.

Discussion

Insulin is secreted from isolated islets of Langerhans when the islets are immersed in a medium containing a high-glucose concentration. Concurrently, somatostatin receptors become recruited to the islet surface, where they can be detected with radiolabelled somatostatin (Mehler et al., 1980). We have previously shown that new somatostatin receptors probably originate from the secretory granule membranes, which must make contact with the plasma membrane during exocytotic release of hormone (Mehler et al., 1980; Sussman et al., 1982). The present studies provide further support for this hypothesis, since we can now distinguish the somatostatin receptor recruitment event from that of insulin secretion itself. We find that insulin secretion but not somatostatin-receptor recruitment is blocked by replacement of chloride in the incubation medium by the impermeant anion isethionate. By contrast, phenothiazine drugs, such as TFP and PMTHZ, block both receptor recruitment and hormone secretion.

The mechanism of isethionate blockade of secretion is not fully known, but the evidence at hand

Fig. 4. Scatchard analysis of effect of glucose concentration, TFP or PMTHZ on number and affinity of somatostatin receptors in stimulated islets

(a) The symbols 30R_e and 300R_e on the horizontal axis indicate the concentrations of total binding sites in islets incubated in either 30 or 300mg of glucose/dl respectively. The slopes of the two curves are indicated by the symbols 30K_e and 300K_e, and are essentially identical. (b) The receptor concentration is shown for islets incubated in buffer containing 300mg of glucose/dl (i.e. 300R_e). PMTHZ and TFP are shown to decrease the number of somatostatin receptors recruited (indicated by PMTHZ K_e and TFP K_e) to the islet surface by incubation in 300mg of glucose/dl. By contrast the affinity constants of the receptors were identical in 300mg of glucose/dl (300K_e), TFP (TFPK_e) or PMTHZ (PMTHZ K_e).
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indicates that it may act primarily at the fission step of exocytosis. Isethionate blocks not only insulin secretion, as reported by Somers et al. (1980) and Orci & Malaisse (1980), but also exocytosis form other systems, including chromaffin cells (Pollard et al., 1979, 1981), parathyroid cells (Brown et al., 1978) and macrophages (Weissman et al., 1978). At a subcellular level, isethionate blocks chemiosmotic lysis of chromaffin granules by competitively inhibiting Cl\(^-\) influx driven by an ATP-dependent electrogenic influx of protons (Pazoles et al., 1980). This chemiosmotic lysis process in granules has been found to parallel the secretion of adrenaline from chromaffin cells in many ways. This has led to the proposal of an osmotic theory of secretion involving extracellular Cl\(^-\) permeation of the complex formed between granules and plasma membranes before fission (Pollard et al., 1979, 1981). Of necessity, the relationship of isethionate action in islets to other systems remains indirect. But if the isethionate action on islets is indeed focused primarily on the fission step, then however it works, one would not expect the preliminary contact event to be modified. As anticipated, somatostatin-receptor recruitment is unaffected in the presence of isethionate, even though glucose-induced insulin secretion is blocked.

Phenothiazine blockade of both somatostatin-receptor recruitment and insulin secretion was not particularly surprising. TFP is known to block insulin secretion, and some have presumed it to work by action on calmodulin, supposing that calmodulin in some way mediated Ca\(^{2+}\) action and membrane contact before secretion (Gagliardino et al., 1980; Valverde et al., 1981; Janjic, 1981). Watkins & Cooperstein (1983) have recently reported that calmodulin promotes the binding of inside-out \(\text{^{125}I}\)-labelled plasma membranes to secretion granules. Furthermore these workers have demonstrated a dose-dependent effect of trifluoperazine in inhibiting this calmodulin-induced binding.

However, the potency of PMTHZ, compared with that of TFP, is inconsistent with calmodulin as a target for the phenothiazines, and even excludes a trivial, local anaesthetic action as a mechanism of action of these drugs. For both inhibition of calmodulin (Levin & Weiss, 1976) and local anaesthetic effects (Poste & Reeve, 1972), the \(K_i\) values for TFP and PMTHZ are approx. 1–10 and 350\(\mu\)M respectively. By contrast, synexin is sensitive to both drugs in the 1–10\(\mu\)M range.

Synexin is widely distributed in tissues (Creutz et al., 1982) and it specifically induces Ca\(^{2+}\)-dependent contact between chromaffin granules (Creutz et al., 1978). Synexin binds specifically to secretory granules and plasma membranes (Pollard et al., 1981; Scott et al., 1983), and also promotes fusion between aggregated granules (Creutz, 1981). We are thus led to conclude that the target of phenothiazine drugs in islets may also include synexin or a synexin-like factor.

However, whatever the site of action of these drugs, it remains clear that there is a very close correlation between somatostatin-receptor recruitment and hormone secretion over a wide range of drug concentrations, as indicated in the correlation plot in Fig. 3. These data indicate that glucose-induced insulin secretion indeed appears to coincide with recruitment of somatostatin receptors derived from secretory-granule membranes upon contact between granules and plasma membranes just before fission. Blockade of fission, as with the isethionate-drug condition, does not interfere with contact. Yet blockade of contact, as in the phenothiazine-drug condition, precludes fission and hormone release.

The location of somatostatin receptors on the external granule surface indicates that the receptor site must pass across a hydrophilic bilayer in some manner to be detected from outside the cell. This is consistent with the observation that the time course for receptor recruitment is somewhat slower than for insulin secretion (Draznin et al., 1982). Such a kinetic character may have physiological significance, since occupation of the receptor by somatostatin may be a mechanism for turning off insulin secretion later.

Examples of location of plasma-membrane receptors on the cytoplasmic surfaces of secretory organelles are now becoming more common (Tanabe et al., 1979). There is now additional evidence suggesting the presence of other hormone receptors in association with secretion granules. Investigating prolactin cells and somatotrophic cells from the rat anterior pituitary, Rosenzweig & Kanwar (1982) have reported significant localization of dopamine (3,4-dihydroxyphenethylamine) to subcellular organelles involved in hormone secretion, including the Golgi cisternae and immature and mature secretory granules. Similarly, Hazum et al. (1982) observed the localization of two labelled analogues of gonadotropin releasing hormone (gonadoliberin) to the cell surface membrane and also to secretory granules and smooth membranes in close vicinity to the Golgi complex. These reports are providing morphological support for the concept of certain hormone receptors being associated with secretion granules. If this is indeed the case, then this methodological approach for studying certain intracellular events of exocytosis (i.e. granule fusion to the surface membrane and granule fission with hormone discharge) may be applicable in other tissues.

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