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Effect of Somatostatin on Insulin Secretion Induced by Ionophore A23187[1] (39636)

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Somatostatin, a hypothalamic peptide initially reported to suppress pituitary growth hormone secretion (1), has been found to possess extra-pituitary effects. The secretions of gastrointestinal hormones such as gastrin (2) and secretin (3) have been reported to be inhibited by somatostatin as have the release of the pancreatic glucoregulatory hormones, insulin (4), and glucagon (5). The ability of somatostatin to inhibit hormone secretion in various endocrine tissues has prompted the suggestion that this peptide may act on a common step in secretory processes (6).

Calcium ion is recognized as an essential requirement for the secretion of most, if not all, peptide hormones (7) and recent evidence suggests that somatostatin's inhibition of glucose-induced insulin release may be related to the divalent cation (8, 9). In an effort to explore the relationship between somatostatin and calcium further, we have investigated the inhibitory action of somatostatin on glucose and ionophore A23187-induced insulin secretions in the isolated, perfused dog pancreas.

Methods and materials. Pancreases were obtained from mongrel dogs (17-25 kg) and perfused according to procedures previously described (10). Normal perfusate consisted of Krebs-Ringer bicarbonate buffer containing 4% dextran and 75 mg% glucose. The ionic concentrations of this medium in mEq/liter are: Na^+, 143; K^+, 5.9; Ca^{2+}, 2.5; $H_2PO_4^-$, 1.2; Mg^{2+}, 1.2; Cl^-, 125.8; SO_4^{2-}, 1.2; and HCO_3^-, 24.6. Cyclic somatostatin (Bachem, Inc.) and ionophore A23187 (generously supplied by Dr. Robert Hamill, Eli Lilly and Co.) were added as required. A23187 was dissolved in ethanol before being added to the desired perfusate (11). Insulin was determined by a modification (11) of the coated charcoal immunoassay procedure described by Herbert *et al.* (12). Interference caused by A23187 was corrected by preparing standard curves containing the drug. The Student's two-sample *t* test was used for all statistical comparisons.

Each pancreas was initially perfused for 20 to 30 min with normal perfusate to establish a basal insulin secretory rate before addition of either 300 mg% glucose or 10 μM A23187. In experiments designed to test the effect of somatostatin, the peptide was introduced at -15 min and continued until the completion of the ionophore experiments (-15 to 30 min) or the first 25 min of the glucose experiments (-15 to 25 min).

Results and discussion. Elevation of the ambient glucose concentration has long been recognized as the principal stimulus for insulin secretion by the pancreatic beta cell. The typical biphasic pattern of secretion induced by this sugar (300 mg%) is illustrated in Fig. 1 (solid line). First phase release consists of a spike of secretion (minutes 0 to 6) while second phase is represented by an increasing rate of secretion. This type of secretory pattern, while characteristic for glucose-induced insulin release, differs from that elicited by ionophore A23187. In the presence of A23187 (10 μM), insulin secretion was stimulated in a monophasic pattern with a peak secretory rate occurring at about minute 12 (Fig. 2, solid line). This is in good agreement with a previous communication from this laboratory (11) where the ionophore response was found to be completed by minute 30 despite continued presence of the drug.

Although structurally unrelated and diverse with regard to stimulated insulin release, both secretagogoues were sensitive to inhibition by somastatin. As reported by others (4), somatostatin (100 ng/ml) was

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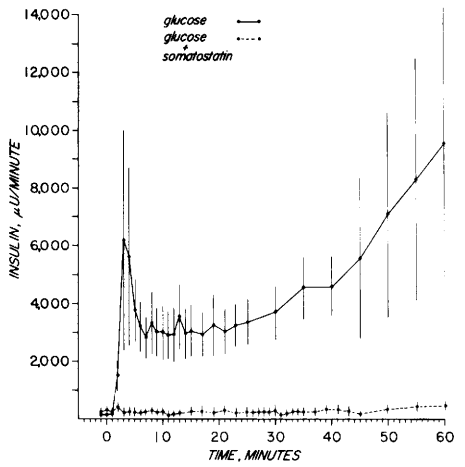

FIG. 1. Insulin secretion induced by 300 mg% glucose (solid line; $N = 6$) and inhibition by somatostatin (dashed line; $N = 5$). Somatostatin (100 ng/ml) was present from −15 to 25 min. Each point represents the mean ± SEM.

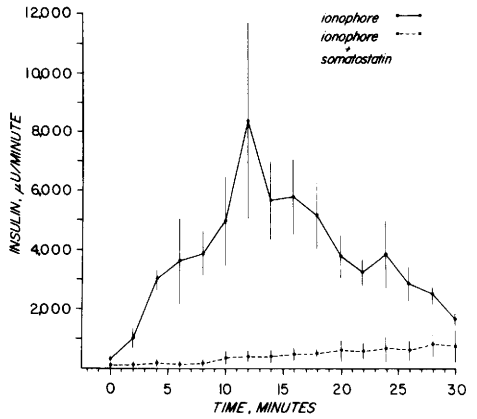

FIG. 2. Insulin secretion induced by 10 μM A23187 (solid line; $N = 6$) and inhibition by somatostatin (dashed line; $N = 4$). Somatostatin (100 ng/ml) was present from −15 to 30 min. Each point represents the mean ± SEM.

noted to suppress both phases ($P < 0.05$) of glucose-induced insulin secretion (Fig. 1, dashed line). Although the peptide was discontinued at minute 25, insulin secretion was suppressed throughout the 60-min perfusion, clearly indicating the potent inhibitory effect of the compound. Insulin secretion elicited by ionophore A23187 was also abolished ($P < 0.05$) in the presence of somatostatin with the secretory rate never rising above 800 μU/min (Fig. 2, dashed line). These results are in sharp contrast to the paradoxical increase in insulin secretion noted by Fujimoto and Ensinck (13) in rat islet cultures following administration of both somatostatin and A23187. This variance in data could possibly be due to differences in the concentrations of calcium and ionophore A23187 employed in the two studies (2.5 mEq/liter of Ca^{2+} and 10 μM A23187 compared to 1.8 mEq/liter of Ca^{2+} and 19.1 μM[2] ionophore for Fujimoto and Ensinck), or might be attributable to either differences in the species of β-cells tested, or to differences in the preparations used in the two investigations.

An increase in intracellular calcium in response to an appropriate stimulus is believed to be a necessary prerequisite for the initiation of insulin release (14). Recent reports have indicated that the inhibitory effect of somatostatin on glucose-induced secretion can be reversed by supraphysiological quantities of calcium, suggesting that somatostatin may act by inactivation of some calcium dependent event in the secretory process (8, 9). The insulinotropic action of ionophore A23187 in the perfused dog pancreas can be theorized to be due to the ability of this compound to elevate intracellular calcium, possibly by transporting the ion from the extracellular fluid. In support of this theory, we have previously demonstrated that ionophore-induced insulin secretion in the dog pancreas preparation is dependent upon an adequate perfusate calcium concentration (11). While conclusive data regarding somatostatin's mechanism of action is not currently available, we feel the present findings support the concept that somatostatin may act by uncoupling some calcium-related event critical to the insulin secretory process.

Summary. Cyclic somatostatin, at a concentration which inhibited both phases of glucose-induced insulin release, completely suppressed monophasic insulin secretion elicited by ionophore A23187. The ability of A23187 to initiate insulin secretion is believed to be highly dependent upon the drug's capacity to influence calcium transport. Thus, the present findings suggest that

[2] Calculated from the concentration of 10 μg/ml reported by Fujimoto and Ensinck.

the negative influence of somatostatin on the insulin secretory process may be exerted on some calcium-related event critical to the release mechanism.

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