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Does somatostatin inhibition of insulin secretion involve two mechanisms of action?

(calcium/differential sensitivities/dose-response/dual function)

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ABSTRACT **Somatostatin, the hypothalamic growth hormone release inhibitory factor (GHRIF), directly inhibits both the first and second phases of insulin secretion. The sensitivities of these two phases of insulin secretion to somatostatin differ remarkably. The first phase of secretion is approximately 25 to 50 times more sensitive to somatostatin inhibition than is the second phase. In addition, somatostatin inhibition of insulin secretion during the second phase is "reversed" by supplemental calcium, whereas the somatostatin effect on the first phase is unaffected by additional calcium. These findings suggest that the cellular events which produce the two phases of insulin secretion are separate processes, and that somatostatin has a dual mechanism of action in inhibiting insulin secretion.**

Insulin secretion, both *in vitro* and *in vivo*, in response to a sustained glucose stimulus is characterized by a diphasic response (1). This diphasic response consists of an initial rapid secretory phase followed by a decline in rate of secretion and then a slowly rising secretion rate. Somatostatin (GHRIF) has been shown to inhibit both the first and second phases of insulin secretion (2–4). A dose–response relationship for somatostatin inhibition of the second phase has been demonstrated (3), but no such dose–response relationship has been shown for somatostatin inhibition of the first phase. The relative sensitivities of these two phases to somatostatin inhibition have not been studied.

Calcium ion (Ca^{2+}) is essential for insulin secretion (5), as it is for many other secretory processes (6). Increasing Ca^{2+} concentration increases the amount of insulin released in response to a glucose stimulus during both the first and second phases of secretion by the isolated perfused rat pancreas (1, 5). Somatostatin inhibition of insulin secretion during the second phase is reversed by elevating the Ca^{2+} concentration (7). Whether elevation of the Ca^{2+} concentration will antagonize somatostatin inhibition of the first phase has not been reported. The purpose of this paper is to report a marked difference in the somatostatin concentration required for inhibition of these two phases of secretion and also to report that the ability of an elevated Ca^{2+} concentration to reverse the somatostatin inhibition of these two phases is strikingly different.

METHODS

The technique used for perfusing the isolated rat pancreas was the same as that previously described (1). The perfusion medium was as that previously described except for the addition of Mg^{2+} at 2.4 meq/liter. Since elevation of Mg^{2+} counteracts the effect of Ca^{2+} (8), its presence may be responsible for the somewhat lower amounts of insulin released when compared to those reported previously (1, 5). All animals used were male rats of the Sprague-Dawley strain weighing about 300 g and fasted 20–24 hr before use. Insulin radioimmunoassay was carried out by the Grodsky and Forsham method (9). Three series of experiments were performed. The first series of experiments involved stimulation of insulin secretion by elevating the glucose concentration to 300 mg/dl in the arterial supply to the pancreas for 60 min. In the middle of this stimulation somatostatin was introduced by a constant rate infusion pump to produce the desired concentration in the arterial supply, i.e., it was infused during the middle 20 min of the 60 min stimulation by glucose (preceded and followed by 20 min of control glucose stimulation without somatostatin). When the ability of elevated Ca^{2+} to reverse somatostatin inhibition was tested (7) somatostatin was infused for 30 min and Ca^{2+} was elevated during the middle 10 min of this period; again the total period of glucose stimulation was 60 min. Control experiments were carried out in which Ca^{2+} alone was elevated during the glucose infusion in the absence of somatostatin.

The second series of experiments involved stimulation by glucose (300 mg/dl) for 40 min. In these experiments somatostatin had been added to the perfusing medium prior to the onset of perfusion at concentrations of somatostatin of either 2 or 5 ng/ml.

The third series of experiments was designed to test whether a dose–response relationship exists for somatostatin inhibition of first phase insulin release. These experiments involved double square wave pulses of glucose to produce a glucose concentration of 300 mg/dl of perfusate, separated by a rest period of 10 min. The glucose pulse was of 3 min duration, and when somatostatin was used, its infusion preceded the beginning of glucose stimulation by 2 min, and was continued for 1 min beyond the glucose pulse. For each concentration of somatostatin there were four preparations, and each preparation served as its own control, having one glucose pulse with somatostatin and one glucose pulse without somatostatin. Since successive glucose pulses do not necessarily produce an identical amount of insulin release, but the amount of insulin released may decline with successive pulses (10), the sequence of glucose alone first, or glucose plus somatostatin first, was alternated from one preparation to the next to randomize this potential source of variation. When the ability of Ca^{2+} to block somatostatin inhibition of the first phase was tested, the Ca^{2+} concentration was elevated simultaneously with the infusion of somatostatin.

The somatostatin used was the synthetic cyclic compound synthesized by the solid-state method by Yamashiro and Li (11), who also supplied somatostatin with formyl-blocked tryptophan and to whom the authors are indebted for generous supplies of both compounds.

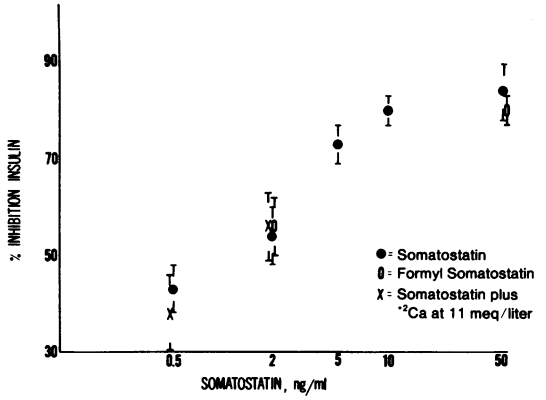

FIG. 1. This graph plots the percent inhibition of insulin release during the first phase as a function of the log of the somatostatin concentration in the perfusate. On the same graph are shown percent inhibition by somatostatin with formyl-blocked tryptophan at concentrations of 50 ng/ml and at 2 ng/ml. In addition is shown the lack of effect of elevating Ca^{2+} at the two lowest concentrations of somatostatin. For each point the standard error is indicated.

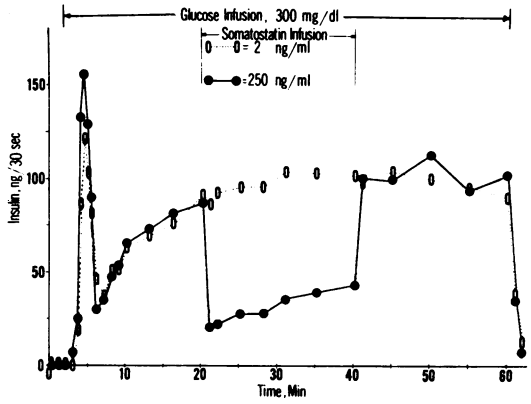

FIG. 2. This graph shows that 2 ng/ml of somatostatin infused in the middle of a 60 min glucose stimulation of the isolated pancreas does not inhibit insulin release in the second phase. By contrast, 250 ng/ml of somatostatin produces approximately 70% inhibition of insulin release. Each curve represents the average insulin secretion by three pancreas preparations. Standard errors are omitted for the sake of easier readability of the graph.

RESULTS

Dose–response relationship

Fig. 1 shows the percent inhibition‡ of insulin release during the first phase by somatostatin in concentrations ranging from 50 to 0.5 ng/ml. What is striking is that 2.0 ng/ml produces approximately 55% inhibition, whereas we have previously shown that it takes approximately 50 ng/ml to produce a comparable inhibition when administered in the middle of the second phase (4). The next larger dose, 5 ng/ml, produces approximately 75% inhibition (compare with Fig. 2 where 250 ng/ml produces approximately 70% inhibition of the second phase).

Data shown in Fig. 2 demonstrate that 2 ng/ml of somatostatin has no inhibitory effect on the second phase, whereas somatostatin at 250 ng/ml produces an inhibition of approximately 70% ($P = 0.05$). Thus, there is an approximate 25- to 50-fold difference in sensitivity of the first and second phases of insulin release to the inhibitory effect of somatostatin, with the first phase being more sensitive.

Fig. 1 also shows the effect of [formyl-Trp]somatostatin at dose levels of 2 and of 50 ng/ml. The inhibitory effect of this compound does not differ from that of the unmodified somatostatin molecule, indicating that the unmodified tryptophan *per se* is not essential for this biological activity of somatostatin.

Fig. 3 shows the time course of glucose-induced insulin release when somatostatin was either present in, or absent from, the perfusion medium throughout the entire experiment including the equilibration period. Somatostatin at 2 and 5 ng/ml inhibits insulin release during the first phase to a degree quite comparable to that demonstrated in Fig. 1, e.g., at 2 ng/ml the inhibition is approximately 50% ($P < 0.02$) and at 5 ng/ml the inhibition is approximately 65% ($P < 0.01$). On the other hand, the data clearly show no inhibition of release during the second phase at either of these somatostatin concentrations ($P > 0.1$).

Fig. 4 shows the time course of insulin release for the experiments shown in Fig. 1. In addition, data from 24 experiments with insulin release in response to glucose alone are shown. This graph shows the dose–response effect as in Fig. 1, but more importantly, it demonstrates the fact that with increasing concentrations of somatostatin, not only is the initial release of insulin delayed, but also the peak release occurs later in time.

Calcium effects

Fig. 5 demonstrates the potentiation of insulin release during the second phase by elevating Ca^{2+} from the control level of 4.5 meq/liter to 11 meq/liter. This potentiation amounts quantitatively to an extra insulin release of 0.8 μg when compared to the average of the two 10 min periods preceding and following the period of Ca^{2+} elevation. In our previous report (7), in which Ca^{2+} elevation was shown to counteract somatostatin inhibition, comparable potentiation was also shown. Specifically, when Ca^{2+} was elevated to 8 meq/liter, the potentiation was 0.5 μg, and when elevated to 11 meq/liter, the potentiation was 0.8 μg.

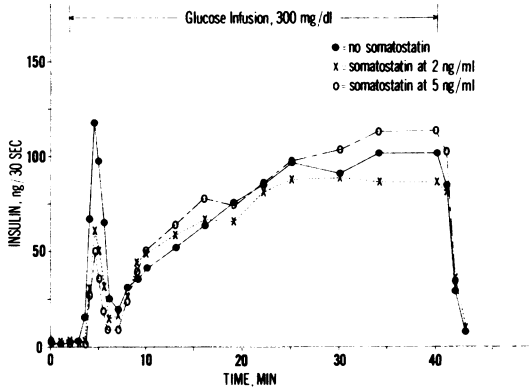

FIG. 3. In the experiments portrayed in this graph somatostatin was present both during the equilibration period and throughout the period of stimulation. The control experiments with no somatostatin represent the average of four different pancreas preparations and experiments with two different somatostatin concentrations are the average of three preparations each. The ability of low concentrations of somatostatin to inhibit the first phase of insulin release and their lack of effect upon the second phase of insulin release are clearly apparent. Standard errors are omitted for the sake of easier readability of the graph.

‡ % Inhibition = 100 × [total release (TR) with glucose alone − TR with glucose plus somatostatin]/TR with glucose alone.

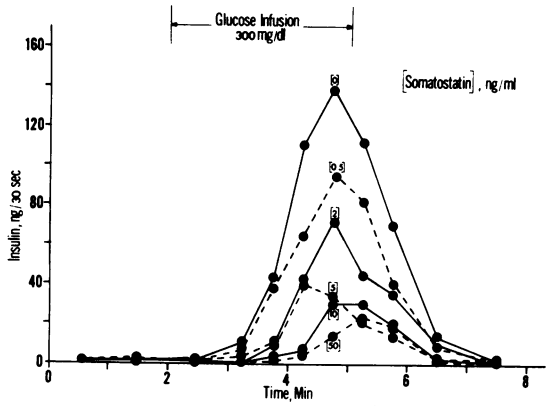

FIG. 4. This graph shows the time course of insulin release at the various somatostatin concentrations shown in Fig. 1. Each curve where somatostatin was present shows the average insulin release by four different pancreas preparations. The control curve (zero somatostatin) is the average of 24 control experiments. Standard errors are omitted for the sake of easier readability of the graph.

Fig. 1 also shows the failure of Ca^{2+} elevation to counteract somatostatin inhibition of the first phase. Ca^{2+} was elevated to 11 meq/liter at the two lowest concentrations of somatostatin used, i.e., 0.5 ng/ml and 2 ng/ml, respectively. In no case did the amount of insulin released differ significantly from the amount released when Ca^{2+} was at its normal concentration ($P > 0.1$), i.e., no reversal of somatostatin inhibition occurred.

DISCUSSION

The rapid first phase of insulin release probably reflects the release of stored granules lying adjacent to the beta cell membrane. If this is the true explanation of the rapid initial first phase, then this membrane phenomenon is remarkably sensitive to somatostatin inhibition. In the experiments illustrated in Figs. 1 and 4, somatostatin was present before the glucose stimulus was delivered. The facts that both the onset of insulin release and the peak insulin release are delayed as a function of increasing somatostatin concentration are consistent with the view that somatostatin may be competing with glucose for the same membrane receptor site or sites from which somatostatin is displaced by glucose.

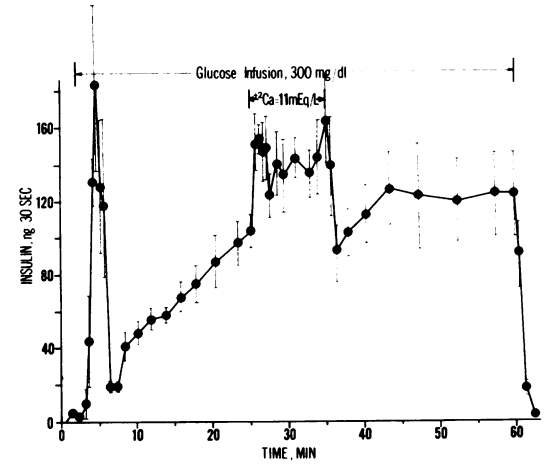

FIG. 5. This graph demonstrates that elevating Ca^{2+} in the middle of a glucose infusion will potentiate insulin secretion. The data presented are from four different preparations and show the mean and standard error at each point in time.

The differential sensitivity of the two phases of insulin release to somatostatin was an unexpected observation, as well as the fact that Ca^{2+} "reversal" of somatostatin inhibition could not be demonstrated for the first phase. These observations strongly suggest that somatostatin has a dual action on the insulin secretory process. (1) Competitive binding to a membrane glucose receptor and/or inhibition of metabolic events which lead to initiation of the first phase of insulin secretion. This is not reversed by elevating Ca^{2+} concentration and is extremely sensitive to somatostatin. (2) Possible competitive binding to a glucose receptor which is related to initiation of intracellular events leading to the second insulin secretory phase and/or direct inhibition of a metabolic process leading to the second phase of release. Insulin release during this phase is potentiated by elevated Ca^{2+} in either the presence or absence of somatostatin and this release process is much less sensitive to somatostatin inhibition.

An alternative explanation for the difference in sensitivities as presented in Fig. 3 is the fact that the phenomenon of tachyphylaxis or tolerance could account for these observations. This explanation cannot be excluded by the data shown in Fig. 3. It is, however, eliminated by the experiments shown in Fig. 2, where somatostatin was introduced only during the second phase. Even under these conditions, when tolerance could not have occurred, the low concentration of somatostatin (2 ng/ml) which inhibited the first phase by approximately 50% was ineffective in the second phase.

These data lead to the inescapable conclusion that the phases of insulin release are two separate processes. They may be influenced or initiated by "different" glucose receptors. If one assumes that somatostatin inhibits the first phase insulin release via some membrane receptor, and if the second phase of release were controlled by the same receptor, one would expect that the first and second phases would exhibit the same sensitivity to somatostatin. Since a 25- to 50-fold difference in sensitivity was observed, one must postulate separate processes. Even if somatostatin activity is not associated with a membrane receptor one must still conclude that the release processes are separate.

These data also suggest that somatostatin inhibition of insulin secretion cannot be explained exclusively by inactivation of calcium, although a competitive inactivation by somatostatin of a Ca^{2+}-initiated process during the second phase may occur.

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