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# Selective Inhibition of Glucagon and Insulin Secretion by Somatostatin Analogs[1] (40393)

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Somatostatin (SRIF) has been shown to inhibit release or secretion of a variety of hormones including glucagon, insulin, gastrin and growth hormone. For therapeutic purposes it might be desirable to achieve selectivity in the inhibition of one or the other hormones. Towards this end, analogs of SRIF have been synthesized and tested both in *in vivo* and *in vitro* systems. The synthetic modifications include (a) substitution of a D form for the L-amino acids, (b) removal or addition of one or more amino acid(s) from the C-terminal or N-terminal end, (c) replacement of amino acids with other L-amino acids, and (d) alteration in cyclic structure. SRIF and linear SRIF have been found to have similar inhibitory activities (1), while bicyclo SRIF inhibits only growth hormone release and has no effect on insulin or glucagon release (2).

In order to be therapeutically useful in diabetes, SRIF should ideally block pancreatic glucagon release without inhibiting insulin release. It should not affect other hormones, except potentially diabetogenic hormones. Brown *et al.* (3) showed that $DTrp^8$ SRIF is 8–10 times more potent than native (unsubstituted) SRIF in inhibiting growth hormone, glucagon and insulin release. They further showed that [$DCys^{14}$] as well as [$DTrp^8$, $DCys^{14}$] SRIF were more potent in inhibiting arginine induced glucagon release than insulin release *in vivo* in rats (4). Meyers *et al.* (5) confirmed these findings and extended them to include [$Ala^2$, $DCys^{14}$].

In the present investigation, we used the isolated rat pancreas perfusion system to define the effect of 10 SRIF analogs on glucagon and insulin secretion, particularly with regard to their capacity to selectively inhibit glucagon secretion. We have previously shown that perfusion of isolated pancreas gives reproducible responses to various stimulators and inhibitors of both insulin and glucagon release (6).

*Materials and methods.* In our study, we evaluated ten somatostatin analogs: [$DCys^{14}$], [$DCys^3$], [NTyr, $DTrp^8$], [$DTrp^8$], [$Ala^2$, $DTrp^8$], [(5F$Trp^8$)], [CGln(Gly)$_2$, $DTrp^8$], [$DTrp^8$, $DCys^{14}$], [$Ala^2$, $DTrp^8$, $DCys^{14}$], [$Ala^2$, $DCys^{14}$] and somatostatin itself for effects on glucagon and insulin release in an isolated rat pancreas system. Fed, male, Sprague-Dawley rats (Flow Research Animals) weighing 250–350 g were used. The pancreas was isolated and perfused according to the method of Curry *et al.* (7). The perfusion media consisted of Krebs-Henseleit bicarbonate buffer, pH 7.4, containing 3% Dextran T-40 (Pharmacia) and 1% human serum albumin (Cutter Laboratories). The buffer was gassed with 95% $O_2$–5% $CO_2$ for 15 min prior to and throughout the perfusion. The pancreas was perfused for 10 min with 5.5 m*M* glucose alone to stabilize insulin and glucagon release at low levels, and then 19.2 m*M* arginine, a potent stimulator of both glucagon and insulin secretion, was added for the remaining 45 min of perfusion. Somatostatin (SRIF) or somatostatin analogs at concentrations of 10 ng/ml or 1 ng/ml were introduced after 15 min arginine perfusion (during the second phase of hormone release), continued for 15 min, and then shut off for the remaining 15 min. Flow rate of the unrecirculated buffer was maintained at 6–7.5 ml/min and effluent was continuously collected at one minute intervals. Insulin (IRI) and glucagon (IRG) release were measured by radioimmunoassays and expressed as mean μU IRI/ml/min or ng IRG/ml/min, respectively (6). The arginine stimulated portion of each perfusion was divided into three 15-min periods designated preanalog (I), an-

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alog (II) and postanalog (III) phase.

*Results.* Prior to testing SRIF analogs, the control arginine perfusion system was defined. Our findings are in accord with our previously published data (6). As can be seen in Fig. 1, arginine stimulated perfusions show a maximal stimulatory effect on both insulin and glucagon release during the first 15 min (phase I). Mean hormone release concentrations/ml of phases II and III were compared to the maximal stimulation of phase I within each perfusion. Insulin secretion was 80% during phase II and 49% during phase III (both significant at $P > .01$) compared with phase I. Glucagon secretion was 84% during phase II and 69% during phase III (not significant).

In Fig. 2, the sequence of SRIF and the analogs tested are shown. Fig. 3 shows the effects of SRIF or SRIF analogs introduced during phase II of arginine stimulation compared to phase I of each perfusion. It may be seen that at 10 ng/ml, SRIF reduced insulin release to 41% of control and glucagon release to 9% of control. At 1 ng/ml, SRIF effects were less marked, with insulin release reduced to 79% of control and glucagon release reduced to 39% of control. In order to determine the significance of the effects of SRIF analogs compared to SRIF effects, $P$ values were calculated by unpaired $t$ test. Of the 10 analogs tested at 10 ng/ml concentration, 8 analogs [DCys$^3$; DCys$^{14}$; Ala$^2$, DCys$^{14}$; DTrp$^8$, DCys$^{14}$; CGln(Gly)$_2$, DTrp$^8$; NTyr, DTrp$^8$; Ala$^2$, DTrp$^8$; (5FTrp)$^8$] showed *glucagon suppression similar to SRIF* ($P$ = *n.s.*). Ala$^2$, DTrp$^8$, DCys$^{14}$; and DTrp$^8$ were less effective than SRIF ($P < .01$).

The effects on insulin release at a concentration of 10 ng/ml for 5 of the 10 analogs NTyr, DTrp$^8$; DTrp$^8$; Ala$^2$, DTrp$^8$; (5FTrp$^8$); and CGln(Gly)$_2$, DTrp$^8$; were not significantly different from SRIF. Analogs DCys$^3$; DCys$^{14}$; Ala$^2$, DCys$^{14}$; DTrp$^8$, DCys$^{14}$; and Ala$^2$, DTrp$^8$, DCys$^{14}$; all showed *lesser inhibitory effects on insulin release than SRIF* ($P < .05$ to $P < .01$). With the possibility in mind that at lower concentrations, greater differential effects might be apparent, the 5 analogs that showed differential effects on insulin release at 10 ng/ml were then tested at 1 ng/ml concentration and compared with SRIF. In addition, CGln (Gly)$_2$ DTrp$^8$ was also tested. At this concentration, DCys$^3$; DCys$^{14}$; and CGln(Gly)$_2$, DTrp$^8$ showed significantly less inhibitory effect on insulin release when compared with SRIF ($P < .05$ to $P < .01$). In addition, DCys$^{14}$; DTrp$^8$, DCys$^{14}$; Ala$^2$, DTrp$^8$, DCys$^{14}$; and CGln (Gly)$_2$, DTrp$^8$ showed significantly enhanced gluca-

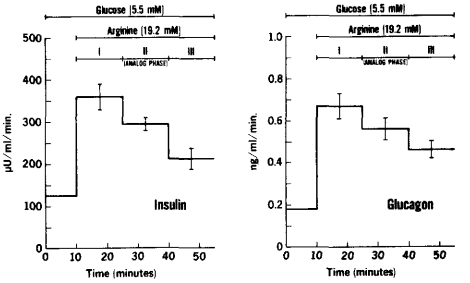


FIG. 1. Arginine perfusions ($N = 14$) showing typical insulin and glucagon responses to 19.2 m$M$ arginine stimulation. In each perfusion, phase I served as an internal control (100% stimulation), with phase II (analog) and phase III (post analog) compared to I.

| | |
|---|---|
| Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys | Somatostatin |
| Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-DCys | DCys$^{14}$ |
| Ala-Gly-DCys-Lys- Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys | DCys$^3$ |
| Tyr-Ala-Gly-Cys-Lys-Asn-Phe-Phe-DTrp-Lys-Thr-Phe-Thr-Ser-Cys | NTyr, DTrp$^8$ |
| Ala-Gly-Cys-Lys-Asn-Phe-Phe-DTrp-Lys-Thr-Phe-Thr-Ser-Cys | DTrp$^8$ |
| Ala-Ala-Cys-Lys-Asn-Phe-Phe-DTrp-Lys-Thr-Phe-Thr-Ser-Cys | Ala$^2$, DTrp$^8$ |
| Ala-Gly-Cys-Lys-Asn-Phe-Phe-5FTrp-Lys-Thr-Phe-Thr-Ser-Cys | (5FTrp)$^8$ |
| Ala-Gly-Cys-Lys-Asn-Phe-Phe-DTrp-Lys-Thr-Phe-Thr-Ser-Cys-Gln-Gly$_2$ | CGln(Gly)$_2$, DTrp$^8$ |
| Ala-Gly-Cys-Lys-Asn-Phe-Phe-DTrp-Lys-Thr-Phe-Thr-Ser-DCys | DTrp$^8$, DCys$^{14}$ |
| Ala-Ala-Cys-Lys-Asn-Phe-Phe-DTrp-Lys-Thr-Phe-Thr-Ser-DCys | Ala$^2$, DTrp$^8$, DCys$^{14}$ |
| Ala-Ala-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-DCys | Ala$^2$, DCys$^{14}$ |

FIG. 2. Structural modifications of the somatostatin molecule are indicated by the underlined amino acids. Analog designations used in the text are listed in adjacent column.

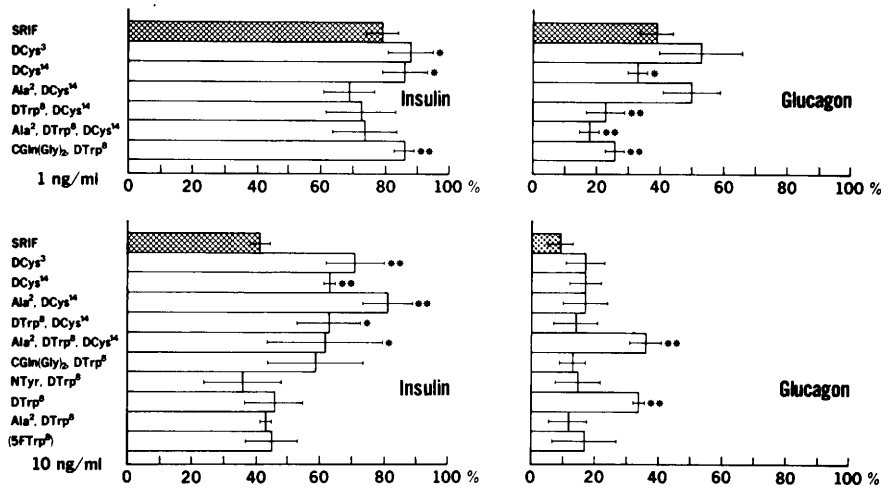


FIG. 3. Effects of SRIF analogs infused in phase II expressed as per cent of phase 1 mean hormone release. *P* values were calculated for effects of analogs compared to effects of SRIF for insulin and glucagon at both concentrations (** $P < .01$, * $P < .05$). Number of perfusions: $N = 3$ for 1 ng/ml; $N = 2$ for 10 ng/ml for SRIF analogs. $N = 4$ for 1 ng/ml; $N = 3$ for 10 ng/ml for SRIF.

gon suppression ($P < .05$ to $P < .01$).

It would appear from evaluation of data shown in Fig. 3 that at 1 ng/ml, analogs DCys$^{14}$; DTrp$^8$, DCys$^{14}$; Ala$^2$, DTrp$^8$, DCys$^{14}$; and CGln (Gly)$_2$, DTrp$^8$ show a dissociation of insulin–glucagon suppressive effects when compared to SRIF. In the case of Ala$^2$, DCys$^{14}$; and Ala$^2$, DTrp$^8$, DCys$^{14}$, the insulin effects are unchanged and the glucagon effects are enhanced. In the case of DCys$^{14}$ and CGln (Gly)$_2$, DTrp$^8$, the insulin effects are less and the glucagon effects are greater than that found with SRIF.

At 10 ng/ml, the major evidences for dissociation are decreases in insulin suppression with no change in glucagon suppression, and in one case, DTrp$^8$, decreased glucagon suppression with unchanged insulin effects.

Analyses of insulin and glucagon release during phase III of arginine stimulation, when SRIF or analogs were removed from the perfusion media, in most cases, showed that when hormone release had been significantly inhibited during phase II, hormone release tended to rise during phase III. When release was not significantly affected during phase II, release tended to fall, comparable to the decrease in hormone levels found in phase III of arginine control perfusions.

*Discussion.* Our observations indicate that modifications of cysteine residues or of the C terminal end of somatostatin resulted in loss or decrease of inhibitory effects of SRIF on insulin release. Additional modifications at Trp$^8$ to the D form enhanced the effect on glucagon suppression by C terminal modified analogs when tested at 1 ng/ml. Modifications at the Trp$^8$ position alone or in combination with modifications at the *N* terminal end or at Ala$^2$ position resulted in analogs which were either equipotent or less potent than SRIF.

Observations similar to these have been documented by others. Using DCys$^{14}$; DTrp$^8$, DCys$^{14}$; and Ala$^2$, DCys$^{14}$; Brown *et al.* (4) and Myers *et al.* (5) have shown that in *in vivo* systems these analogs exhibit greater potency on glucagon suppression than on insulin. Substitution of Ala$^2$ resulted in a molecule equipotent with SRIF on both insulin and glucagon suppression *in vivo* (8). The DTrp$^8$ modification, when tested in vivo (9), resulted in a molecule 8–10 times more potent than native SRIF on both insulin and glucagon secretion.

In attempts to clarify the differential effects of SRIF analogs on insulin–glucagon release, different investigators have tested a variety of analogs on *in vivo* and *in vitro* systems. Effendic *et al.* (10), using a rat pancreas perfusion, showed that des Ala$^1$, Gly$^2$; alone or in combination with des Asn$^5$; resulted in abolition of glucagon suppression with maintenance of insulin suppression. Sarantakis *et al.* (11) reported similar results with des Ala$^1$, Gly$^2$; Asn$^5$; *in vivo*. Elimination of inhibitory

effects on both insulin and glucagon secretion was seen with a des $Ala^1$, $Gly^2$ compound in which the cysteine bridges were replaced by nonsulfur linkages (12). Similar loss of potency was seen with conversion of SRIF to a homoletic cyclic disulfide tetradecapeptide (2). In the latter instance, potency could be restored by substitution with $DTrp^8$.

Our data with various substitutions and deletions on the SRIF molecule show that inhibitory effects on insulin and glucagon release can be dissociated. The mechanism of interaction of SRIF and its analogs with receptors of the $\alpha$, glucagon producing cells, or $\beta$, insulin producing cells, is not known, but our data suggests that there are significant differences in the receptors on these cells. It would appear that an unaltered C terminal of SRIF may be required for binding of SRIF to the $\beta$ cells of the islets of Langerhans. Possibly this relates to the formation of an intra-chain disulfide with $Cys^3$.

Several of the analogs tested warrant further study, especially the $DCys^{14}$; $DTrp^8$, $DCys^{14}$; $Ala^2$, $DTrp^8$, $DCys^{14}$; and CGln $(Gly)_2$, $DTrp^8$ modifications, since these analogs compared with SRIF have the same or lesser effects on insulin secretion and are more potent inhibitors of glucagon release.

*Summary.* The effects of somatostatin and 10 structural analogs of SRIF on arginine stimulated glucagon and insulin secretion were studied in isolated rat pancreas perfusions. All analog data were compared with the inhibitory effects of SRIF. It was observed that structural modifications could produce clear cut dissociation of the inhibitory effects on glucagon vs. insulin secretion. Four compounds which seem to have little effect on insulin release, but compared with SRIF have an enhanced inhibitory effect on glucagon release, are of potential interest in the therapy of diabetes. These compounds are $DCys^{14}$; $DTrp^8$, $DCys^{14}$; $Ala^2$, $DTrp^8$, $DCys^{14}$; and CGln $(Gly)_2$, $DTrp^8$ somatostatin.

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