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Inhibition of Insulin Secretion *in vitro* by Somatostatin *

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The isolated and perfused rat pancreas was used to study the insulin secretion stimulated by 16.7 mM glucose in the presence of SRIF (somatotropin release inhibiting factor); at a concentration of 2 μg/ml, the latter was a potent inhibitor of insulin secretion. Since SRIF is effective in inhibiting growth hormone and glucagon secretion also, the secretory mechanism of the three hormones may have a common basis.

The inhibitory action of somatostatin (SRIF = somatotropin release inhibiting factor) on the secretion of growth hormone has been confirmed by a number of works (2, 5, 9, 14). While studying this effect, is was found that SRIF produced hypoglycemia *in vivo*, explicable in terms of a decrease in hepatic glucose production. Furthermore, the intravenous inyection of SRIF resulted in a lowering of the basal plasma insulin and glucagon levels also (12), and insulin returned to initial values more slowly than the glycemia (7). These effects have been confirmed by ALBERTI *et al.* (1), IVERSEN (6), SAKURAI *et al.* (12) and LEBLANC *et al.* (8).

This communication presents the preliminary results of the perfusion of the isolated rat pancreas with SRIF and 16.7 mM glucose used to study the inhibitory action of somatostatin on glucose-induced insulin release.

Materials and Methods

In the present experiments, the isolated perfused rat pancreas was used as described by GRODSKY *et al.* (4) and SUSSMAN *et al.* (13), without any adjacent organs such as stomach or spleen (3). Arterial flow was achieved by cannulation of the aorta and the perfusate was freely collected from the portal vein, without recirculation, at intervals of 2 minutes. The perfusion fluid consisted of a Krebs Ringer's bicarbonate buffer (pH = 7.35) supplemented with 0.5 % albumin and equilibrated with 95 % O_2 and 5 % CO_2. A constant-flow-rate of 2.5 ml/min was

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achieved with a perfusion pressure of about 15-25 mm Hg. The temperature was adjusted to 38° C.

SRIF * was dissolved in 16.7 mM glucose (final concentration 2 μg/ml) and perfused for 40 minutes after an initial perfusion period with 2.7 mM glucose.

At a flow rate of 2.5 ml/min at the end of each perfusion an additional pulse injection of 0.2 ml/min of glucose (50 % w/v) was administered, to test the insulin response.

Insulin was measured immunologically (10) with our own antibody Ab-38 at an 1/60,000 dilution.

In all the experiments the means are given with the corresponding SEM.

Results

Figure 1 shows the mean insulin secretion in the isolated perfused rat pancreas. The upper part represents the dynamics of insulin secretion stimulated with 16.7 mM glucose after an initial period of perfusion with 2.7 mM glucose. The lower panel shows the results of perfusion with 16.7 mM glucose plus SRIF (2 μg/ml), also after an initial perfusion period with 2.7 mM glucose. As can be seen, in the presence of SRIF, 16.7 mM glucose is uncapable of stimulating insulin secretion; the latter remains steady, at levels not significantly different from the baseline, throughout the test. After stopping the SRIF perfusion and switching to 2.75 mM glucose, the inhibition persists, even in the presence of such a potent stimulus as a pulse of concentrated glucose (0.2 ml of 50 % glucose in one minute).

Figure 2 shows the total insulin secretion in ng/hour. While in nine perfusions with 16.7 mM glucose the mean was 926.84±90.73 ng/hour, in the five perfusions with 16.7 mM glucose plus SRIF (2 μg/ml) it was 223±20 ng/hour. The

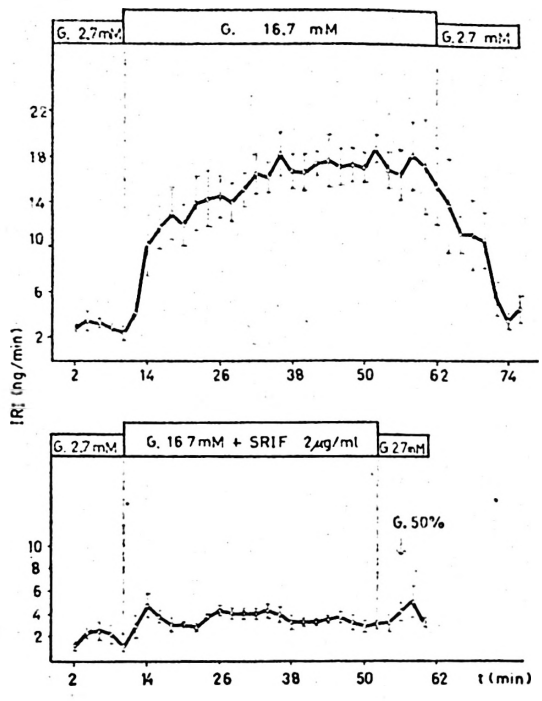

Fig. 1. *Insulin release of the isolated and perfused rat pancreas following 16.7 mM plus SRIF (lower part).*

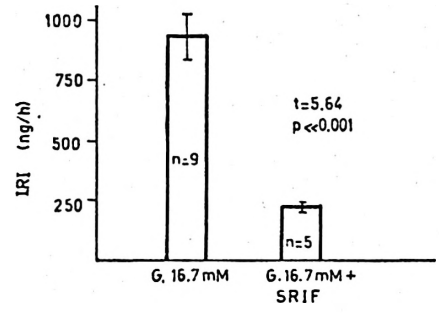

Fig. 2. *Total Insulin (ng/hour) released with 16.7 mM glucose and 16.7 mM glucose plus SRIF.*

difference is highly significant ($t = 5.64$; $p \ll 0.001$).

Discussion

The results show that SRIF is a potent inhibitor of insulin secretion *in vitro*, since

* SRIF was kidly supplied by Dr. SCHALLY, University of Tulana (U.S.A.).

the inhibition is apparent when SRIF is perfused together with a very effective concentration of glucose (16.7 mM) and after stopping the SRIF perfusion and switching to 2.75 mM glucose, the action of a pulse of concentrated glucose was also inhibited. These results are in good agreement with preliminary ones reported *in vitro* by OSORIO *et al.* (11) and ALBERTI *et al.* (1). The mechanism of this inhibitory action is not known, but the fact that HGH, insulin and glucagon secretion are inhibited simultaneously seem to indicate that these three hormones are secreted by mechanisms with a common basis.

Resumen

Utilizando páncreas aislado y perfundido de rata se ha estudiado la secreción de insulina estimulada por glucosa 16,7 mM. En presencia de SRIF (factor inhibidor de la liberación de somatostatina) a una concentración de 2 μg por miligramo se produce una potente inhibición de la secreción de insulina. Ya que esta sustancia es un potente inhibidor de la hormona del crecimiento y del glucagón, parece que las tres hormonas son segregadas por mecanismos con una base común.

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