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Inhibition of the Glucose Induced Insulin Release by Somatostatin in the Isolated Perfused Rat Pancreas. Action of Cyclic AMP, Glucagon and Glibenclamide

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Insulin release in the perfused isolated rat pancreas was measured after stimulation with 16.5 mM glucose with and without somatostatin (cyclic form, 100 ng/ml) in the medium. A complete blockage of the typical biphasic pattern of insulin release ocurred with somatostatin in the medium. Such blockage was abolished when cAMP (2.5 mM) and a 0.5 ml solution of glucagon (1 mg/ml) were continuously perfused for 20-minute periods and for 30-second periods correspondently. It did not take place when glibenclamide (HB-419) was perfused for a 20-minute period at a rate of 10 μg/ml.

The results suggest that the adenylcyclase dependent mechanisms of glucose-induced insulin release are involved in the inhibition of the glucose-induced insulin secretion by somatostatin.

As it has been demonstrated, somatostatin, a 14 aminoacid polypeptide isolated from the hypothalamus and synthesized later (4, 5, 7, 21), inhibits growth hormone release in acromegalics (3, 13) and in normal humans (26). Apart from eliciting similar effects on other polypeptide hormones (15, 19, 22), it acts as a strong inhibitor of the glucose-induced insulin release in man (1, 19) and in animal (1, 10, 20, 25), and also blocks the insulin release induced by arginine (6) and alanine (22).

The action of somatostatin on such different hormones suggets the involvement of a common mechanism.

In a previous paper (20), we pointed towards the possibility of such a mechanism being the adenyl-cyclase system. The present study was undertaken to further explore such a possibility.

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Materials and Methods

Twenty-nine overnight-fasted male rats, weighing between 250-300 g were used throughout the experiments.

The pancreas was prepared according to the technique described by GRODSKY *et al.* (12) and SUSSMAN *et al.* (23), and was isolated from all the surrounding organs, such as stomach and spleen, according to FUSSGANGER *et al.* (11). Once all the vessels were ligated, the thorax was opened and the aorta was immediately cannulated. The perfused liquid was freely collected from the portal vein without recirculation at intervals of two minutes. The flow rate was adjusted to 2.5 ml/min, which resulted in a perfusion pressure of 20-40 mm Hg.

The perfusion fluid consisted of Krebs-Ringer-Bicarbonate buffer (pH = 7.38) supplemented with 2 % albumin (Behrinwerke AG, Marburg/Lahn) and was continuously gasified with a mixture of 95 % O_2 and 5 % CO_2. The isolated organ was placed in a perfusion chamber at a constant temperature of 38° C.

The pancreases were allowed to stabilize for a 20-minute period before starting to collect the effluent.

Insulin was measured according to MELANI *et al.* (18).

Dibutyryl cyclic AMP was purchased from Boehringer (Inhelm, Mannheim). Insulin free glucagon was supplied by Novo (Copenhagen); glibenclamide (HB-419) was a kind gift of Boehringer (Mannheim) and somatostatin was used in the synthesized cyclic di-sulfide form (Merck, Sharp, Dohmey).

cAMP *, glucagon and glibenclamide were infused through the isolated perfuser rat pancreas using a Braun-Perfusor apparatus during 20 min, 30 sec and 20 min respectively.

* cAMP = Abbreviation for dibutyryl adenosin 3:5-cyclic monophosphate.

Results

Figure 1 shows the two phases of insulin release that are known to occur when 16.5 mM glucose is continuously infused through the isolated rat pancreas. When somatostatin (100 ng/ml) was present in the perfusion medium (fig. 2) a complete inhibition of both phases resulted. When the insulin release induced by 16.5 mM glucose was blocked by somatostatin (100 ng/ml), the presence of cAMP in the perfusion medium at a concentration of 2.5 mM during a 20-minute period resulted

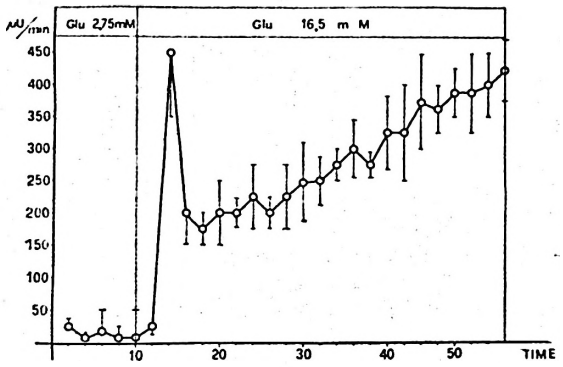

Fig. 1. *Time course of insulin release in response to glucose (16.5 mM) stimulation in the isolated perfused rat pancreas.* The flow rate was adjusted at 2.5 ml/min. Vertical bars represent mean ± SEM from five experiments.

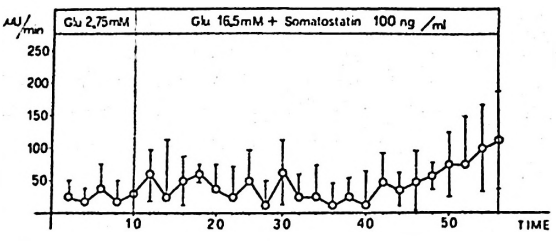

Fig. 2. *Time course of insulin release in response to glucose (16.5 mM) stimulation when somatostatin (100 ng/ml) was co-infused through the isolated perfused rat pancreas.* The flow rate was adjusted at 2.5 ml/min. Vertical bars represent mean ± SEM from five experiments.

in a slow but steady increase of the insulin levels (fig. 3). This increase reached, from the 26th minute onward, the same degree of insulin output achieved during the second phase of the insulin release pattern induced by 16.5 mM glucose alone figure 1).

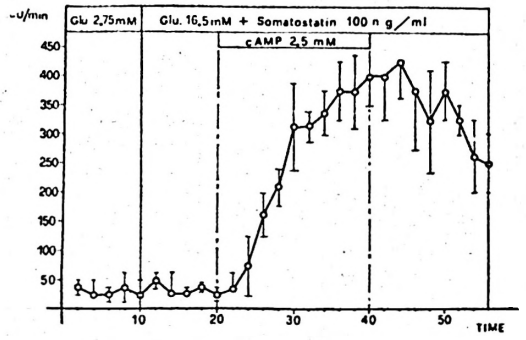

Fig. 3. *Effect of cAMP (2.5 mM) infused through the isolated perfused rat pancreas when glucose (16.5 mM) induced insulin release was blocked by somatostatin (100 ng/ml).*
The flow rate was adjusted at 2.5 ml/min. Vertical bars represent mean ± SEM from five experiments.

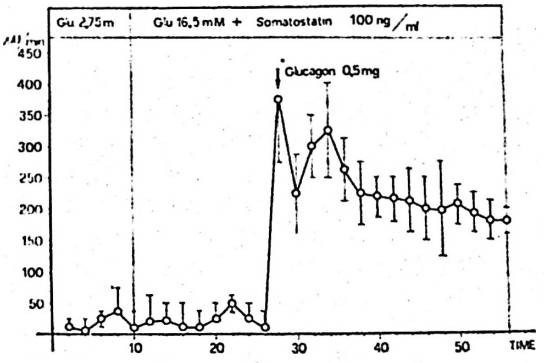

Fig. 4. *Effect of glucagon (0.5 mg) infused for a 30-second period on the 26th minute through the isolated perfused rat pancreas, when glucose (16.5 mM) induced insulin release was blocked by somatostatin (100 ng/ml).*
The flow rate was adjusted at 2.5 ml/min. Vertical bars represent mean ± SEM from five experiments.

The insulin blockage achieved by somatostatin (100 ng/ml) was then challenged by glucagon (0.5 ml of a 1 mg/ml solution) during a 30-sec period (fig. 4). A sharp biphasic increase in the insulin release pattern was observed.

Being the pancreas stimulated by glucose 16.5 mM, the co-infusion of glibenclamide at a rate of 10 μg/ml from the 20th min up to the 40th min (fig. 5) resul-

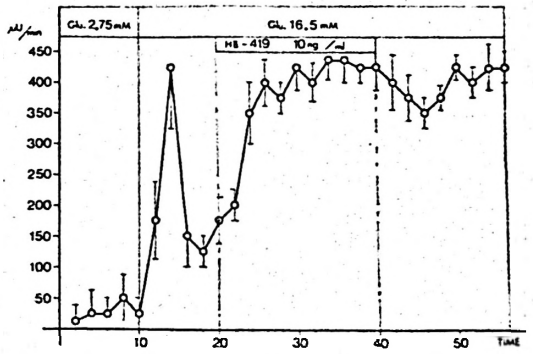

Fig. 5. *Effect of glibenclamide (10 μg/ml) infused for a 20-minute period, when the insulin secretion was stimulated by glucose (16.5 mM) infused through the isolated rat pancreas.*
The flow rate was adjusted at 2.5 ml/min. Vertical bars represent mean ± SEM from four experiments.

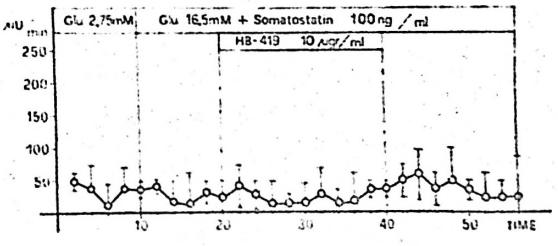

Fig. 6. *Effect of glibenclamide (10 μg/ml) infused for a 20-minute period through the isolated rat pancreas, when glucose (16.5 mM) induced insulin release was blocked by somatostatin (100 ng/ml).*
The flow rate was adjusted at 2.5 ml/min. Vertical bars represent mean ± SEM from five experiments.

ted in a net increase over the insulin release levels achieved when glucose 16.5 mM was infused alone (figure 1). However when somatostatin (100 ng/ml) was blocking the insulin release induced by glucose 16.5 mM, the co-infusion of glibenclamide (10 μg/ml) was unable to overcome somatostatin blocking action (figure 6).

Discussion

It has been previously reported that the inhibitory action of somatostatin on the glucose induced insulin release from isolated perfused rat pancreas could be overcome when challenged by a 6-min long pulse of cAMP (2.5 and 5 mM or theophylline (5 mM) (20). These findings point toward the possibility that the increase in the cAMP intracellular levels was responsible for such resumption. To assess further this possibility, cAMP was introduced into the perfusion medium at a concentration of 2.5 mM over a 20-minute period (fig. 3) and glucagon, a known inducer of insulin release (24) throughout the adenyl cyclase system (16), was co-infused (0.5 ml of a 1 mg/ml solution) during a 30-sec period (fig. 4). In both cases the glucose 16.5 mM induced insulin release was blocked by somatostatin (100 ng/ml).

When cAMP was introduced into the medium, the insulin output increased sharply soon reaching the same level achieved during the second phase of the glucose 16.5 mM induced insulin release (fig. 3). When glucagon was co-infused during a 30-sec period (fig. 4), a sharp increase in the insulin levels could be found from the 34th minute onward.

That cAMP, theophylline and glucagon were able to overcome somatostatin action on glucagon-induced insulin release raises the question of whether or not the resumed insulin release was really due to an increase in the cAMP levels, or to some inspecific stimulatory insulin release activity common to the three substances tested, and independent of the presence of somatostatin in the medium. To clarify this point, a powerful potentiator of the glucose induced insulin release, glibenclamide (17) was infused through the isolated pancreas. When glibenclamide was infused at a concentration of 10 μg/ml for a 20-min period, a clear potentiation of the glucose 16.5 mM induced insulin release resulted (fig. 5). However when glucose 16.5 mM induced insulin release was blocked by somatostatin (100 ng/ml) no effect of the glibenclamide (10 μg/ml) potentiating activity on insulin release could be found (fig. 6).

In agreement with these findings is the work done by KANEKO *et al.* (14), who demonstrated that somatostatin inhibited basal and TRH stimulated accumulation of cAMP in rat anterior pituitary glands *in vitro*. In addition to this, the restoration of the first phase of 16.5 mM glucose induced insulin release blocked by somatostatin by increasing the extracellular concentrations of Ca^{++} ions (8) could suggest a somatostatin action at a membrane level.

Whether or not the amount of somatostatin used in the present study (100 ng/ml) is of physiological or pharmacological importance remains to be seen, in view of the studies reported by DUBOIS *et al.* (9), who found appreciable amounts of somatostatin in the pancreas using the immunofluorescence technique, and the data presented by ARIMURA *et al.* (2), who found somatostatin-like immunoreactive activity in sheep serum.

Until a reliable technique is developed to measure somatostatin, there will always be the open question of whether to attribute to somatostatin at the doses currently used, a purely pharmacological action, or to the contrary, a very important role such as a negative regulator of the endocrine glands thus far studied.

Resumen

Se mide la liberación de insulina en páncreas aislado y perfundido de rata en respuesta a estimulación con glucosa 16,5 mM, con o sin somatostatina (forma cíclica, 100 ng/ml) presente en el medio.

Cuando la somatostatina está presente en el líquido de perfusión se observa una inhibición completa del patrón bifásico de liberación de insulina. Esta inhibición desaparece cuando se perfunde con cAMP (2,5 mM) durante un período de 20 minutos y al perfundir durante 30 segundos con 0,5 ml de una solución de glucagón (1 mg/ml), pero no desaparece al perfundir con glibenclamida durante 20 minutos a un ritmo de 10 μg/ml.

Los resultados sugieren que el sistema de la adenilciclasa puede estar involucrado en la inhibición por somatostatina de la secreción de insulina inducida por la glucosa.

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