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SHORT COMMUNICATIONS

Reversal of the inhibitory effects of calcitonin gene-related peptide (CGRP) and amylin on insulin secretion by the 8-37 fragment of human CGRP*

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Abstract—The 8-37 fragment of human calcitonin gene-related peptide [(8-37)hCGRP] antagonizes the effects of calcitonin gene-related peptide (CGRP) and amylin in a number of tissues. We have studied the influence of (8-37)hCGRP on the effects of both CGRP and amylin on insulin secretion. In the perfused rat pancreas, homologous CGRP and amylin, at 75 pM, exerted comparable inhibitory effects on the insulin response to 9 mM glucose (*ca.* 70%; $P < 0.025$). These effects were antagonized by (8-37)hCGRP (1 μM). Our results suggest that CGRP and amylin act on the B-cell, at least in part, through a common receptor.

Calcitonin gene-related peptide (CGRP†) is a 37-amino acid straight chain peptide widely distributed throughout the central and peripheral nervous system, including nerve fibers in the pancreatic islets [1–3]. CGRP exhibits a great homology to islet amyloid polypeptide (IAPP), also called amylin, which has been isolated from amyloid deposits in the islets of Langerhans [4, 5]. Further studies have revealed

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† Abbreviations: CGRP, calcitonin gene-related peptide; (8-37)hCGRP, 8-37 fragment of human calcitonin gene-related peptide.

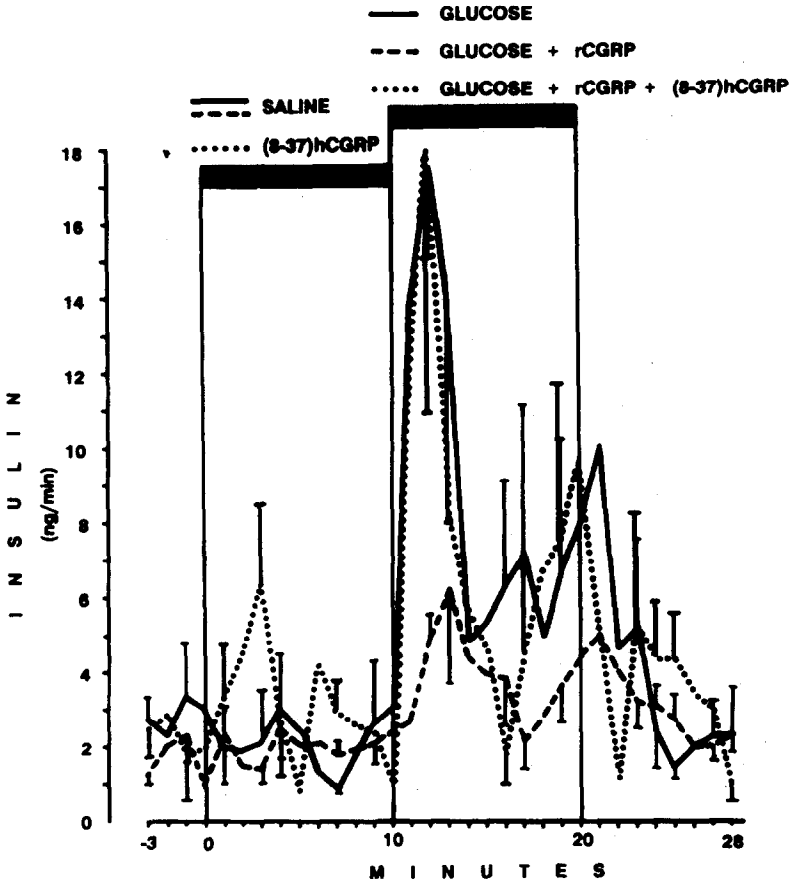

Fig. 1. Reversal of the inhibitory effect of rat-CGRP on glucose-induced insulin release by the (8-37)hCGRP fragment in the perfused rat pancreas. Solid line: basal medium (left panel) followed by glucose (9 mM) infusion (right panel); N = 3. Broken line: basal medium (left panel) followed by glucose (9 mM) + r-CGRP (75 pM) infusion (right panel); N = 6. Dotted line: (8-37)hCGRP (1 μM) infusion (left panel) followed by glucose (9 mM) + r-CGRP (75 pM) + (8-37)hCGRP (1 μM) infusion (right panel); N = 3.

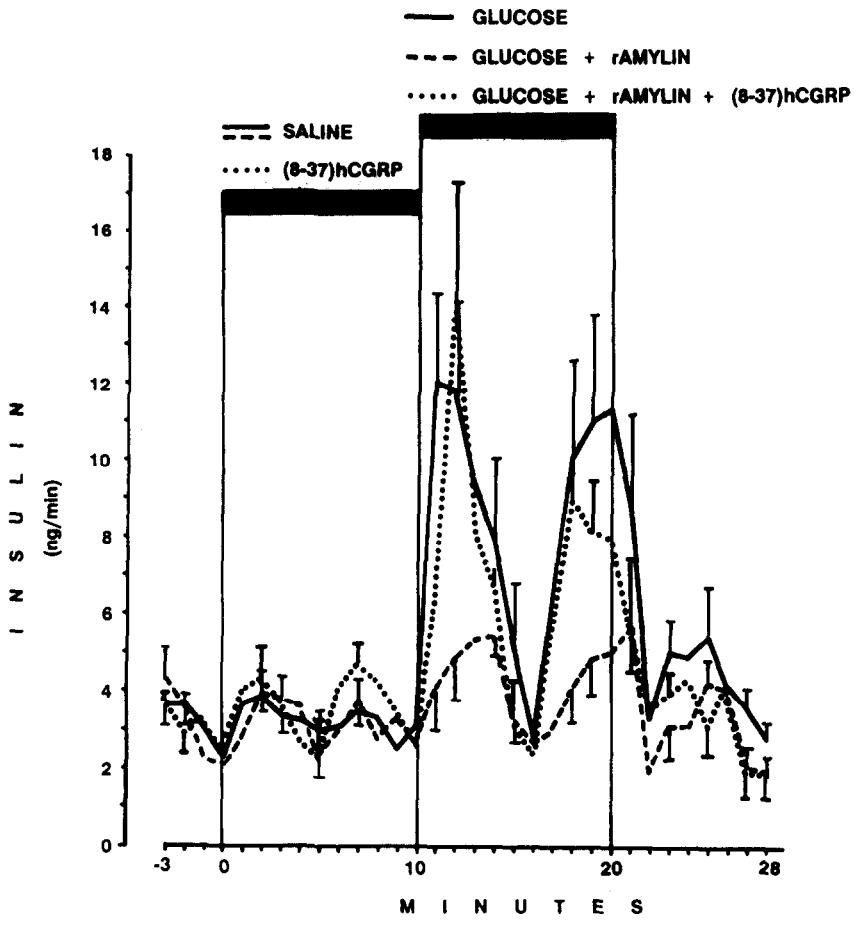

Fig. 2. Reversal of the inhibitory effect of rat amylin on glucose-induced insulin release by the (8-37)hCGRP fragment. Solid line: basal medium (left panel) followed by glucose (9 mM) infusion (right panel); N = 7. Broken line: basal medium (left panel) followed by glucose (9 mM) + r-amylin (75 pM) infusion (right panel); N = 7. Dotted line: (8-37)hCGRP (1 μM) infusion (left panel) followed by glucose (9 mM) + r-amylin (75 pM) + (8-37)hCGRP (1 μM) infusion (right panel); N = 4.

that amylin is present in the pancreatic B-cell secretory granules and that it is co-secreted with insulin [6, 7].

Besides their structural similarities, amylin and CGRP share some biological effects such as hypocalcemic actions [8, 9] and vasodilation [10, 11]. Since the isolation of these two peptides, a great deal of interest has been focused on their effects on pancreatic hormone secretion. Exogenous CGRP has been found to inhibit insulin secretion both *in vivo* and *in vitro* [3, 12]. Concerning the effect of amylin on insulin release, results are controversial [13–16]. In our perfused rat pancreas system, rat amylin consistently suppresses glucose-induced insulin output [17, 18].

Availability of peptide antagonists may contribute to the study of their physiological role. Recently, it has been shown that the 8-37 fragment of human CGRP [(8-37)hCGRP] antagonizes the effects of both CGRP and amylin in a number of tissues, such as liver plasma membranes, skeletal muscle and some vascular beds [19–22].

The present work has been undertaken to investigate the possible influence of (8-37)hCGRP on the inhibition of glucose-induced insulin release evoked by both CGRP and amylin. The study was performed in the perfused rat pancreas.

Materials and Methods

Fed male Wistar rats (200–225 g body weight) were used as donors. After anesthesia of the rat with pentobarbital sodium (50 mg/kg, i.p.), the pancreas was dissected and perfused *in situ* according to the procedure of Leclercq-Meyer *et al.* [23] as adapted in our laboratory [24]. Effluent samples were collected from the portal vein, without recycling, at 1 min intervals (flow rate, 2 mL/min), and frozen at −20° until the time of assay. The perfusion medium consisted of a Krebs–Henseleit buffer (gas phase 95:5, O_2:CO_2; pH 7.4) supplemented with 4% (w/v) dextran T-70 (Pharmacia LKB Biotechnology, Uppsala, Sweden), 0.5% (w/v) Cohn Fraction V bovine serum albumin (Sigma Chemical Co., St. Louis, MO, U.S.A.) and 5.5 mM glucose (Sigma). This medium will be conventionally referred to as basal medium. Synthetic rat CGRP, rat amylin and (8-37)hCGRP (Peninsula Laboratories Inc., Belmont, CA, U.S.A.) were dissolved in 0.9% NaCl containing 0.1% bovine serum albumin (Cohn Fraction V). These solutions were prepared daily, immediately before experiments. It must be mentioned that (8-37)CGRP of human origin was used due to the unavailability of the rat fragment. After a 35-min

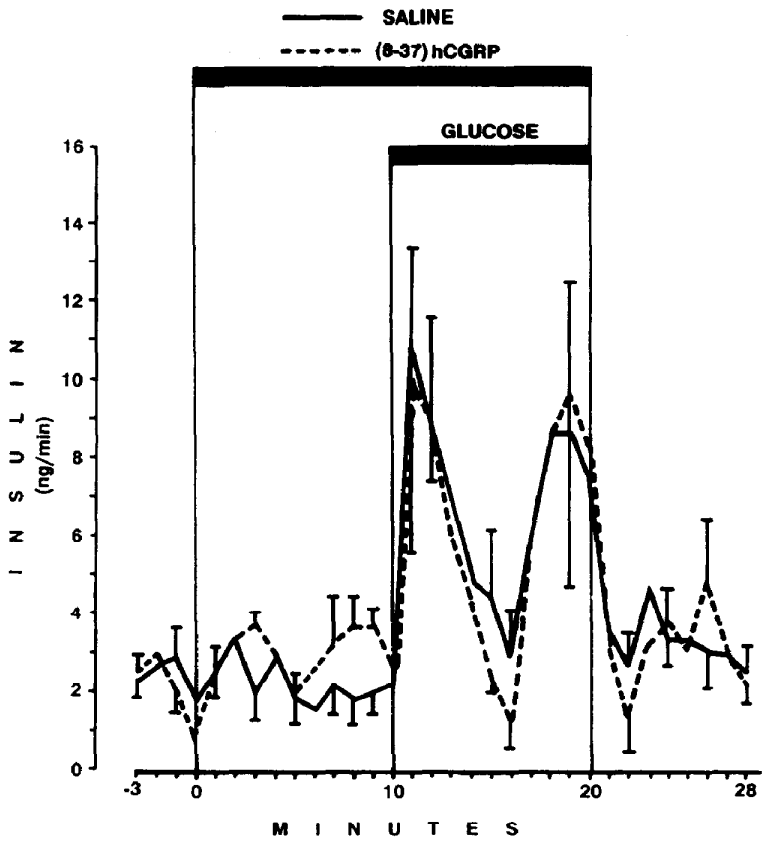

Fig. 3. Effect of (8-37)hCGRP on basal as well as on glucose-induced insulin release in the perfused rat pancreas. Solid line: basal medium (left panel) followed by glucose (9 mM) infusion (right panel); N = 5. Broken line: (8-37)hCGRP (2 μM) infusion (left panel) followed by glucose (9 mM) + (8-37)hCGRP (2 μM) infusion (right panel); N = 4.

equilibration period, baseline samples were collected for 4 min. At zero time, normal saline, containing 0.1% bovine serum albumin, with or without 1 μM (8-37)hCGRP, was infused for 10 min. From 10 to 20 min, the effect of 75 pM CGRP or 75 pM amylin on glucose-induced insulin release was studied both in the presence and in the absence of 1 μM (8-37)hCGRP. In another series of experiments, we examined the effect of 2 μM (8-37)hCGRP on basal as well as on glucose-induced insulin release. Insulin was measured by radioimmunoassay [25]. All samples for each series of experiments were analysed in the same run. Results are presented as the mean ± SEM. Hormone response was calculated as the integrated area of the curve above the mean preinfusion level (average of all the baseline levels for each perfusion). Differences between values were tested for significance by the Student's *t*-test for unpaired samples.

Results

In a first series of experiments (Fig. 1), we studied the effect of CGRP (75 pM) on the insulin release evoked by glucose (9 mM) in the presence and in the absence of (8-37)hCGRP (1 μM). CGRP markedly blocked the insulin response to glucose (incremental area: 14 ± 4 vs 55 ± 15 ng/10 min in control experiments; $P < 0.025$). The blocking effect of CGRP on glucose-induced insulin output was clearly counteracted by (8-37)hCGRP. In the presence of CGRP and its antagonist, the insulin response to glucose was comparable to that found with glucose alone (incremental area: 64 ± 30 vs 55 ± 14 ng/10 min; $P = 0.8$). It is of note that during the first period of the perfusion, when the antagonist was infused alone, basal insulin levels showed a tendency to increase, although this rise was not statistically significant.

A similar protocol was followed to study the effect of amylin (75 pM), alone or in combination with (8-37)hCGRP (1 μM), on glucose-induced insulin release (Fig. 2). Amylin markedly reduced the insulin response to glucose (incremental area: 15 ± 5 vs 52 ± 12 ng/10 min in control experiments; $P < 0.025$). This inhibitory effect is comparable to that found by us, using the same dose of CGRP. As for CGRP, when amylin and the CGRP antagonist were simultaneously infused, amylin did not inhibit the insulin response to glucose (incremental area: 41 ± 10 vs 52 ± 12 ng/10 min in control experiments; $P = 0.6$). The infusion of 1 μM (8-37)hCGRP did not significantly modify basal insulin release.

Finally, we examined the effect of infusing (8-37)hCGRP alone on basal and on glucose-induced insulin output (Fig. 3). This compound, at 2 μM, a level twice that employed in the preceding experiments, failed to significantly modify either basal or glucose-induced insulin release.

Discussion

The foregoing results demonstrate that, in the perfused rat pancreas, homologous CGRP markedly blocked the insulin response to glucose. This observation is in agreement with previous work performed *in vivo* [3, 26] as well as *in vitro*, in cultured isolated islets from adult rats [12]. The

CGRP concentration we have tested (75 pM) is, to the best of our knowledge, the lowest effective dose reported to inhibit insulin output.

As expected, rat amylin, at 75 pM, reduced glucose-induced insulin release. This concentration falls within the range of amylin levels measured in the effluent from the perfused rat pancreas [27, 28]. It is of note that, at the concentration employed, the potency of both amylin and CGRP to inhibit insulin secretion in our pancreas preparation appeared to be virtually identical. Since these peptides provoke regional hemodynamic actions [10, 11], it should be commented that, in our experiments, they caused no change in the perfusate flow rate.

Infusion of (8-37)hCGRP counteracted the inhibitory effect of both amylin and CGRP on glucose-induced insulin secretion, thus suggesting that these peptides act on the B-cell, at least in part, through a common receptor. In fact, cross-reactivity of amylin with CGRP binding sites has been recently reported in liver, skeletal muscle and brain membranes from rat [29, 30]. As pointed out by Galeazza *et al.* [29], studies of a putative receptor for amylin might be hampered by the iodination at the C-terminal tyrosyl residue of amylin, given that the C-terminal region of this peptide seems to be important for binding. Recently Bhogal *et al.* [31] have found specific binding sites for amylin in a number of tissues from several species, including the rat, although, so far, no binding has been detected in pancreas.

A concentration of (8-37)hCGRP, twice that used to antagonize the inhibitory effect of exogenous CGRP and amylin, did not significantly affect either basal or glucose-induced insulin release. This observation does not provide evidence for a role of CGRP/amylin in the normal modulation of insulin secretion. However, it should be taken into account that in other tissues, i.e. mammalian cardiac myocytes [32], rat liver plasma membranes [19] and isolated rat soleus muscle [20], the concentration of (8-37)hCGRP capable of antagonizing the effects of CGRP or amylin has been determined to be 10 μM, which is about one order of magnitude higher than that employed in our perfusions.

Although, at present, a physiological role for endogenous CGRP or amylin in the control of insulin secretion cannot be established, the inhibitory effect of picomolar concentrations of these peptides on insulin release reported here would favour this concept. Finally, our observation that (8-37)hCGRP counteracts the inhibitory effect of CGRP and amylin on insulin secretion would allow speculation that a suitable antagonist of these peptides might be considered in the treatment of type 2 diabetes to improve the insulin secretory defect characteristic of this disease.

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