British Journal of Pharmacology (1996) 117, 347-350

Effect of (8-32) salmon calcitonin, an amylin antagonist, on insulin, glucagon and somatostatin release: study in the perfused pancreas of the rat

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1 The 8-32 fragment of salmon calcitonin ((8-32) sCT) has been proposed as a highly selective amylin receptor antagonist.

2 In the present study, we have studied the influence of (8-32) sCT on the inhibitory effect of both amylin and its structural congener, calcitonin gene-related peptide (CGRP), on insulin secretion in the rat perfused pancreas.

3 Both amylin and CGRP, at 75 pM, clearly inhibited glucose-induced insulin release (by 80% and by 70%, respectively). Simultaneous infusion of 10 μM (8-32) sCT reversed the inhibitory effect of amylin (by 80%; $P<0.05$ vs. amylin experiments) but did not significantly affect the inhibition of glucose-induced insulin output elicited by CGRP. Furthermore, at the same concentration (10 μM), (8-32) sCT alone potentiated the insulin response to 7 mM glucose (2.5 fold; $P<0.05$) whilst it did not affect glucagon or somatostatin secretion.

4 The observation that infusion of an amylin antagonist into the rat pancreas potentiates the insulin response to glucose, favours the concept of endogenous amylin as an inhibitor of insulin release.

5 Finally, as an amylin antagonist at the level of the β-cell, (8-32) sCT might be considered of potential interest in experimental and clinical pharmacology.

Keywords: Amylin (islet amyloid polypeptide); calcitonin gene-related peptide; (8-32) salmon calcitonin; insulin; glucagon; somatostatin; rat pancreas

Introduction

Identification of islet amyloid polypeptide (IAPP), a new peptide also called amylin, in the secretory granule of the β-cell has heightened expectations for understanding of the mechanism of insulin secretion (Lukinius *et al.*, 1989; Porte & Kahn, 1989; Gaeta *et al.*, 1994).

Despite considerable effort, there is, as yet, no conclusive evidence of a role of amylin in the regulation of endocrine pancreas function (Fehmann *et al.*, 1990; Nagamatsu *et al.*, 1990 *et al.*, 1990, Silvestre *et al.*, 1990). Recently, amylin has been shown to inhibit glucose-induced insulin output in different *in vitro* systems, such as the rat perfused pancreas (Dégano *et al.*, 1993; Silvestre *et al.*, 1993), rat perifused islets (Wang *et al.*, 1993), and mouse isolated β-cells (Wagoner *et al.*, 1993). In the perfused pancreas of the rat, short-term infusion of amylin has shown that amylin inhibits the insulin response to secretagogues that act on the β-cell via different mechanisms namely, K and Ca channels, the adenylate cyclase/cyclic AMP system, and phospholipid turnover (Kogire *et al.*, 1991; Salas *et al.*, 1993; Silvestre *et al.*, 1994). It should be emphasized that such an effect has been observed with an amylin concentration (75 pM) comparable to amylin levels found in the effluent of the rat perfused pancreas (Salas *et al.*, 1993; Silvestre *et al.*, 1994). In the same experimental model, pharmacological concentrations of amylin (500 and 750 pM) had no effect on glucagon or somatostatin release (Silvestre *et al.*, 1990; Peiró *et al.*, 1991).

Several groups have failed to detect an insulinostatic effect of amylin *in vitro* (Nagamatsu *et al.*, 1990; O'Brien *et al.*, 1990; Petersson & Ahrén, 1990; Tedstone *et al.*, 1990). Furthermore, in transgenic mice, overexpression of amylin was not associated with reduced insulinaemia (Höppener *et al.*, 1993; D'Alessio *et al.*, 1994; Verchere *et al.*, 1994). On the other hand, a specific amylin receptor in the β-cell has not as yet been characterized (Bhogal *et al.*, 1990).

Availability of peptides which antagonize certain extrapancreatic effects of amylin, i.e. its blockade of insulin-stimulated glucose uptake and glycogen synthesis in muscle (Gaeta *et al.*, 1994), may contribute to determining the influence of amylin on β-cell function. In this context, the 8-37 fragment of amylin antagonizes the inhibitory effect of amylin on insulin release from rat perifused islets (Wang *et al.*, 1993). Similarly, the 8-37 fragment of human calcitonin gene-related peptide (CGRP, a peptide which exhibits a great homology to amylin) counteracts the inhibitory effect of both CGRP and amylin on glucose-induced insulin secretion (Silvestre *et al.*, 1993).

Recently, the 8-32 fragment of salmon calcitonin ((8-32) sCT or AC66) has been proposed as an amylin receptor antagonist which is highly selective for amylin (Young *et al.*, 1992). The present study, performed in the rat isolated perfused pancreas, has been undertaken to explore the ability of (8-32) sCT to curb the inhibition of insulin secretion induced by either amylin or CGRP. We have also tested the effect of (8-32) sCT, in the absence of exogenous amylin, on insulin, glucagon and somatostatin release.

Methods

Fed male Wistar rats (200-225 g body weight) were used as donors. After the rat had been anaesthetized with pentobarbitone sodium (50 mg kg^{-1}, i.p.), the pancreas was dissected and perfused *in situ* according to the procedure of Leclercq-Meyer *et al.* (1976) as adapted in our laboratory

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(Silvestre *et al.*, 1986). Effluent samples were collected from the portal vein, without recycling, at 1 min intervals (flow rate, 2 ml min^{-1}), and frozen at −20°C until the time of assay. The perfusion medium consisted of a Krebs-Henseleit buffer, composition, mM: NaCl 115, KCl 4.7, $CaCl_2$ 2.6, H_2KPO_4 1.19, $MgSO_4.7H_2O$ 1.19 and $NaHCO_3$ 24.9 (gas phase 95 O_2: 5 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 albumin (RIA grade, Sigma Chemical Co., St. Louis, MO, U.S.A.) and glucose 3.2 mM (Sigma).

Synthetic amidated rat amylin (Peninsula Laboratories, Inc., Belmont, CA, U.S.A.), rat CGRP (Peninsula) and (8–32) sCT (a generous gift from Amylin Pharmaceutical Inc., San Diego, CA, U.S.A.) were dissolved in 0.9% NaCl containing 0.1% bovine albumin (Cohn Fraction V). This solution was prepared daily, immediately before experiments. After a 35 min equilibration period, baseline samples were collected for 5 min. The effect of 75 pM amylin or 75 pM CGRP on basal insulin release (from 0 to 5 min) as well as on glucose-induced insulin release (from 5 to 20 min) were studied both in the presence and in the absence of 10 μM (8–32) sCT; additions to the perfusate were performed as described in the corresponding figures. In another series of experiments, we studied the effect of (8–32) sCT on insulin secretion.

Insulin (Yalow & Berson, 1960; Herbert *et al.*, 1965), glucagon (Fallona & Unger, 1974) and somatostatin (Harris *et al.*, 1978) were measured by radioimmunoassay. Anti-pig insulin serum (I8510, Sigma) and rat insulin standards (Novo, Nordisk, Denmark) were employed. Antiglucagon serum (Unger's 30K) and antisomatostatin serum (Unger's 80C) were kindly donated by R.H. Unger (University of Texas Health Sciences Center, Dallas, U.S.A.). All samples for each series of experiments were analyzed in the same run.

Results are presented as the mean±s.e.mean. 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 analysis of variance and by Student's one-tailed *t* test for unpaired samples.

Results

As shown in Figure 1, the insulin response to 7 mM glucose (incremental area: 21.5±4.2 ng 15 min^{-1}) was blocked by 75 pM rat amylin (incremental area: 3.6±4.2 ng 15 min^{-1}; $P<0.025$). The simultaneous infusion of 10 μM (8–32) sCT clearly reversed the insulinostatic effect of amylin (incremental area: 17.5±3.5 ng 15 min^{-1}; $P<0.05$ vs. amylin experiments).

A similar protocol was followed to examine the effect of CGRP, alone or in combination with (8–32) sCT, on glucose-induced insulin release (Figure 2). Infusion of 75 pM CGRP abolished the insulin response elicited by 7 mM glucose (incremental area: 6.2±6.6 ng 15 min^{-1} vs. 23.1±3.2 ng 15 min^{-1} in control experiments; $P<0.05$). The inhibitory effect of CGRP was also observed in the presence of (8–32) sCT (incremental area: 10.9±3.9 ng 15 min^{-1}; $P<0.05$ vs. control experiments).

Finally, we studied the effect of (8–32) sCT alone on basal as well as on glucose-induced insulin release (Figure 3). Infusion of (8–32) sCT failed to modify significantly basal insulin output ($F_{10.90}=1.24$) but clearly potentiated the insulin response to 7 mM glucose (incremental area: 44.4±10 ng 15 min^{-1} vs. 18.6±3.9 ng 15 min^{-1} in control experiments; $P<0.05$).

As illustrated in Figures 4 and 5, respectively, (8–32) sCT did not affect either glucagon or somatostatin release. In both figures, the curves for control and (8–32) sCT experiments overlapped.

Discussion

In agreement with previous results from our laboratory (Silvestre *et al.*, 1993) the foregoing findings show that both amylin and CGRP, at 75 pM, block the insulin response to glucose in the rat perfused pancreas.

As already mentioned, the insulinostatic effect of amylin and CGRP was found to be prevented by simultaneous infusion of (8–37) hCGRP, a putative CGRP antagonist (Silvestre

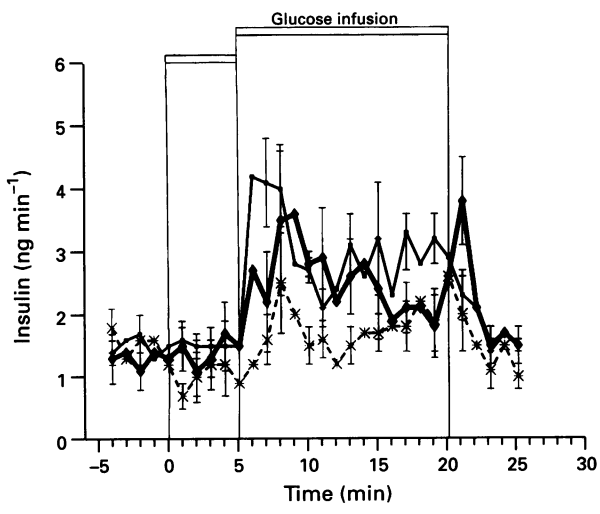

Figure 1 Effect of rat amylin (75 pM) on the insulin response to 7 mM glucose both in the presence and in the absence of 10 μM (8–32) sCT, in the rat perfused pancreas. (■) Control experiments: from 0 to 5 min, saline infusion; from 5 to 20 min glucose infusion ($n=10$); (✱) amylin experiments: from 0 to 5 min, amylin infusion; from 5 to 20 min, glucose+amylin infusion ($n=9$); (◆) (8–32) sCT; experiments: from 0 to 5 min, amylin+(8–32) sCT; from 5 to 20 min, glucose+amylin+(8–32) sCT ($n=7$). Means±s.e.mean.

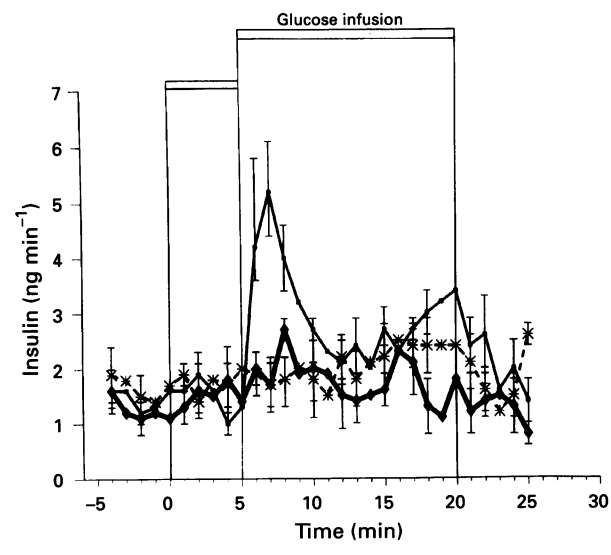

Figure 2 Effect of rat CGRP (75 pM) on the insulin response to glucose (7 mM) both in the presence and in the absence of 10 μM (8–32) sCT, in the perfused rat pancreas. (■) Control experiments: from 0 to 5 min, saline infusion; from 5 to 20 min glucose infusion ($n=10$); (✱) CGRP experiments: from 0 to 5 min, CGRP infusion; from 5 to 20 min, glucose+CGRP infusion ($n=9$); (◆) (8–32) sCT experiments: from 0 to 5 min, CGRP+(8–32) sCT; from 5 to 20 min, glucose+CGRP+(8–32) sCT ($n=7$). Means±s.e.mean.

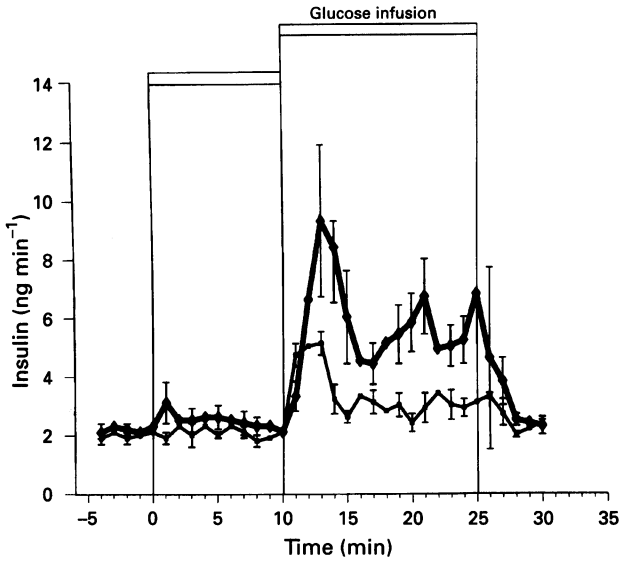

Figure 3 Effect of (8–32) sCT (10 μM) on the insulin response to glucose (7 mM), in the rat perfused pancreas. (■) Control experiments: from 0 to10 min, saline infusion; from 10 to 25 min, glucose infusion ($n=9$); (◆) (8–32) sCT experiments: from 0 to 10 min, (8–32) sCT infusion; from 10 to 25, glucose+(8–32) sCT infusion ($n=10$). Means±s.e.mean.

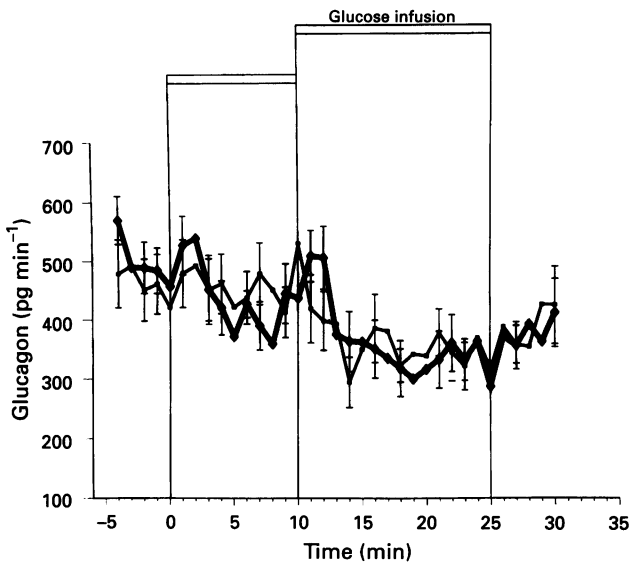

Figure 4 Effect of (8–32) sCT (10 μM) on the glucagon response to glucose (7 μM), in the rat perfused pancreas. (■) Control experiments: from 0 to 10 min, saline infusion; from 10 to 25 min, glucose infusion ($n=7$); (◆) (8–32) sCT experiments: from 0 to 10 min, (8–32) sCT infusion; from 10 to 25, glucose+(8–32) sCT infusion ($n=10$). Means±s.e.mean.

et al., 1993). This observation led to the suggestion that these peptides act on the β-cell, at least in part, through a common receptor (Silvestre *et al.*, 1993). As for (8–37) hCGRP, we have found that the 8–32 fragment of salmon calcitonin, (8–32) sCT, counteracts the inhibitory effect of amylin on glucose-induced insulin release. However, at the same concentration (10 μM), (8–32) sCT did not significantly modify the blunting effect of CGRP on the insulin response to glucose. In this regard, it has been shown that (8–32) sCT selectively inhibited amylin binding in rat brain and was much less potent against CGRP binding (Beaumont *et al.*, 1993). Furthermore, (8–32) sCT was more potent than (8–37) hCGRP in inhibiting the [^{125}I]-amylin binding to nucleus accumbens membranes

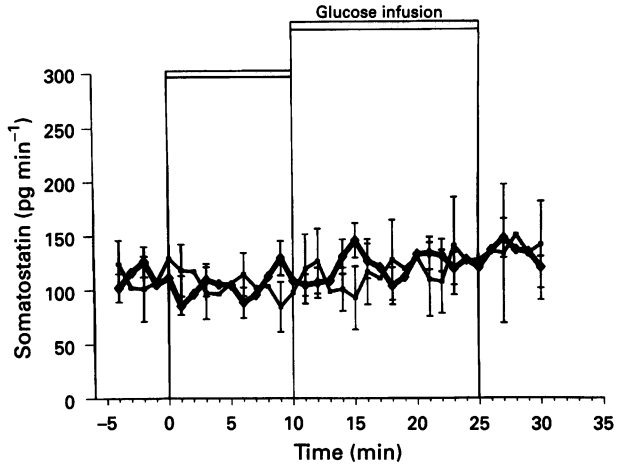

Figure 5 Effect of (8–32) sCT (10 μM) on the somatostatin response to glucose (7 mM), in the rat perfused pancreas. (■) Control experiments: from 0 to 10 min, saline infusion; from 10 to 25 min, glucose infusion ($n=9$); (◆) (8–32) sCT experiments: from 0 to 10 min, (8–32) sCT infusion; from 10 to 25, glucose+(8–32) sCT infusion ($n=10$). Means±s.e.mean.

(Beaumont *et al.*, 1994) and in antagonizing the inhibition of insulin-stimulated glucose incorporation into rat soleous muscle glycogen (Beaumont *et al.*, 1994).

Up till now, no specific receptors for amylin have been identified in the pancreatic β-cell. However, evidence exists for CGRP receptors in β-cell membranes from hamster insulinoma, and amylin can interact with these receptors (Barakat *et al.*, 1993). Our present study indicates that amylin might also act on the β-cell via a receptor other than that of CGRP given that (8–32) sCT effectively reverses the inhibitory effect of amylin on insulin release, while it does not affect the insulin inhibition elicited by CGRP.

Whilst infusion of (8–32) sCT did not affect insulin secretion at 3.2 mM glucose, it approximately doubled the insulin response to 7 mM glucose. Given that (8–32) sCT blocks the inhibition of insulin release caused by exogenous amylin, it can be inferred that this calcitonin fragment antagonizes endogenous amylin, thus potentiating the secretion of insulin. This observation favours the concept of endogenous amylin as exerting a tonic effect on insulin secretion. On the other hand, a direct priming influence of (8–32) sCT on β-cell responsiveness to glucose cannot be ruled out. However, it should be mentioned that, under several experimental conditions, calcitonin does inhibit insulin secretion (Tamarit-Rodríguez *et al.*, 1978; Lunetta *et al.*, 1981; Alwmark *et al.*, 1986).

Finally, (8-32) sCT infusion did not significantly alter glucagon and somatostatin release by the rat perfused pancreas. Accordingly, in the same experimental model, exogenous amylin, at high concentrations (500–700 nM), failed to modify glucagon responses to glucose and to arginine (Silvestre *et al.*, 1990) or the somatostatin response to arginine (Peiró *et al.*, 1991). Thus, the influence of (8-32) sCT on the β-cell does not seem to be mediated by an A- or D-cell paracrine effect.

To sum up, in the rat pancreas (8–32) sCT potentiates insulin release and counteracts the inhibition of insulin output induced by exogenous amylin. Thus, (8–32) sCT may be considered an amylin antagonist at the level of the β-cell, of potential interest in experimental and clinical pharmacology.

This work was supported by grants from the Fondo de Investigación Sanitaria de la Seguridad Social (FIS no. 94/0282 and 93/0184) and the Fundación Eugenio Rodríguez-Pascual, Spain. The expert technical assistance of Ms Pilar García and Ms Encarnación Gutièrrez is gratefully acknowledged. We thank Ms Martha Messman for her secretarial help.

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(Received June 7, 1995
Revised October 6, 1995
Accepted October 10, 1995)