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Effects of Glucagon and Glucagon-Like Peptide-1-(7–36) Amide on C Cells from Rat Thyroid and Medullary Thyroid Carcinoma CA-77 Cell Line

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ABSTRACT

Glucagon is known to stimulate calcitonin secretion by thyroid C cells over a wide range of concentrations, raising the possibility of its interaction with several types of receptors. This study was designed to characterize receptors that mediate the effect of glucagon on a rat C cell line (CA-77).

Binding studies, using radiolabeled [^{125}I]glucagon and [^{125}I]glucagon-like peptide-1-(7–36) amide ([^{125}I]tGLP-1), to CA-77 plasma membranes demonstrated the presence of 1) a glucagon receptor with a dissociation constant (K_d) of 2.3 nM and relative potencies for structurally related peptides as follows: glucagon > oxyntomodulin ≫ tGLP-1; and 2) a tGLP-1 receptor with a K_d of 0.33 nM and relative potencies as follows: tGLP-1 > oxyntomodulin > glucagon. Glucagon stimulated calcitonin secretion from CA-77 cells in a dose-dependent manner over 4 orders of magnitude, with a maximal response of 312% over the basal value and an ED_{50} close to 50 nM. tGLP-1 induced a calcitonin release over 2 orders of magnitude, with a maximal response of 170% over the basal value and an ED_{50} close to 0.2 nM. Glucagon and tGLP-1 stimulated cAMP production in CA-77 cells to similar maximal levels over 4 and 2 orders of magnitude, respectively. The stimulation of cAMP production by glucagon at concentrations over 10 nM was suppressed by the tGLP-1 antagonist exendin-(9–39) amide, whereas the stimulation of calcitonin secretion was only partly abolished. Using a perifusion system of rat thyroid, glucagon and tGLP-1 stimulated calcitonin secretion in a calcium-dependent manner.

It is concluded that glucagon and tGLP-1 receptors are expressed in the rat C cell line (CA-77) and in the normal rat thyroid. The effects of glucagon on calcitonin secretion observed at high concentrations are mediated in part through interaction with tGLP-1 receptors and via an additional non-cAMP-mediated mechanism. (*Endocrinology* **137:** 3674–3680, 1996)

GLUCAGON INFUSION has been reported to produce hypocalcemia in dogs (1), rats (2), pigs (3), and humans (4). At least part of this hypocalcemic effect may be due to the stimulation of calcitonin secretion, as suggested *in vivo* in dogs (5), pigs, sheep (6), and humans (4). *In vitro*, glucagon stimulates calcitonin release by pig thyroid slices (7), trout C cells (8), or rat tumoral C cells (9) in primary culture and by the C cell line rMTC 6–23 (10, 11). However, in most cases, calcitonin secretion is obtained at micromolar concentrations of glucagon, and to date, glucagon receptors have not been characterized on C cells. In contrast, "enteroglucagon" stimulates calcitonin secretion by pig thyroid gland perfused *in situ* at nanomolar concentrations (12). Furthermore, absorption of calcium salts is associated with a prompt rise in plasma enteroglucagon without a change in plasma glucagon in the dog (13). All of these studies were performed before the discovery of the intestinal peptide, glucagon-like peptide-1 [GLP-1-(7–36) amide], also called truncated GLP-1 (tGLP-1) (14). This peptide is coproduced in intestinal L cells (15, 16) and coreleased upon stimulation by luminal nutrients (17–19) with glicentin and oxyntomodulin, both referred to as enteroglucagon (15). Besides its potent glucose-dependent insulinotropic effect, tGLP-1 stimulates somatostatin release in isolated perfused rat stomach (20) and the RIN T3 cell line (21) through interactions with a specific high affinity receptor (22, 23). This receptor was recently described in the rat medullary carcinoma of the thyroid cell line rMTC 6–23 (24). Owing to the low affinity of glucagon and oxyntomodulin for this receptor (21, 25, 26), the effects of glucagon and enteroglucagon on calcitonin secretion described in the literature might be explained in part by interactions of these hormones with tGLP-1 receptor.

The present paper examines the effects of glucagon and tGLP-1 on a C cell line (CA-77 cells) derived from a rat medullary thyroid carcinoma (27) using ligand binding studies, cAMP production, and calcitonin release. Their effects on calcitonin secretion were also investigated using perifused rat thyroid glands.

Materials and Methods

Reagents

Glucagon was obtained from the Novo Research Institute (Bagsvaerd Denmark). Synthetic tGLP-1 and exendin-(9–39) amide were obtained from Peninsula Laboratories (San Carlos, CA). Synthetic human calcitonin was purchased from Ciba-Geigy (Basel, Switzerland). Human/rat oxyntomodulin (OXM) was synthesized in our laboratory (28). 3-Isobutyl-1-methylxanthine (IBMX) and BSA (fraction V) were purchased from Sigma Chemical Co (St. Louis, MO). Mono-[^{125}I]peptides (glucagon, tGLP-1, and calcitonin) and mono-[^{125}I]Tyr-cAMP were obtained by the chloramine-T procedure and purified by reverse phase HPLC on μBondapak C_{18} (Millipore, Milford, MA).

Cell culture and plasma membrane preparations

The CA-77 cell line was obtained from Dr. B. A. Roos (University of Miami, Miami, FL). Cells were plated in a mixture of DMEM-Ham's F-10 medium (1:1; Life Technologies, Eragny, France) supplemented with 10% (vol/vol) FCS, 100 IU/ml penicillin, and 100 μg/ml streptomycin

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and maintained in an atmosphere of 5% CO_2 in air at 37 C. After 2 days of plating, the medium was replaced by serum-free medium constituted by DMEM-Ham's F-10 medium (1:1) supplemented with a mixture of 5 μg/ml insulin, 5 μg/ml transferrin, 5 ng/ml sodium selenite (Sigma), and the antibiotics mentioned above. Serum-free medium was changed at 2- to 3-day intervals, and cells were routinely subcultured weekly after 1:3 dilution. Membranes from CA-77 cells were prepared as previously described (29) and stored at −80 C until use.

Binding studies

Binding experiments with CA-77 membranes were performed in Krebs-Ringer phosphate (KRP) buffer containing 1% BSA and 0.1% bacitracin. Membranes (~40 μg/tube) were incubated in a final volume of 500 μl with approximately 30 pM mono-[^{125}I]tGLP-1 or mono-[^{125}I]glucagon for 45 min at 37 C. The reaction was terminated by the addition of 750 μl ice-cold KRP buffer and centrifuged at 4 C for 5 min at 12,000 × *g*, and the pellets were counted. Nonspecific binding was defined as binding measured in the presence of 0.1 μM tGLP-1 or 1 μM glucagon and was subtracted from total binding.

Calcitonin release and cellular cAMP production

CA-77 cells were grown in 12-well plates (calcitonin release) or in 24-well plates (cAMP measurement) for 6 days, and the medium was changed 1 day before experiments. For calcitonin secretion, CA-77 cells ($0.84 \pm 0.04 \times 10^6$ cells/well; n = 12) were preincubated for 1 h in 1 ml DMEM-Ham's F-12 medium (1 mM Ca^{2+}) containing 1% BSA; incubation was then performed in the same medium, containing aprotinin (200 kallikrein inhibitor units/ml; Biosys, Compiegne, France) and the indicated peptides and calcium concentrations, for 2 h at 37 C. Calcitonin was determined by RIA, as previously described (30). The M732 antibody (goat antiserum raised against synthetic human calcitonin) was a generous gift from Prof. J. M. Garel (University of Pierre et Marie Curie, Paris, France). The detection limit of the assay was 16 pg/tube, with intra- and interassay variations of 5% and 12%, respectively, and the results were expressed as picogram equivalents of human calcitonin. For determination of cellular cAMP production, CA-77 cells ($0.41 \pm 0.02 \times 10^6$ cells/well; n = 10) were washed twice with KRP buffer before the addition of 0.5 ml KRP buffer containing 2% BSA, 0.2 mM 3-isobutyl-1-methylxanthine, and the test peptides. After a 20-min incubation at 25 C, cellular cAMP was extracted, succinylated, and quantified by RIA (31).

Tissue preparation and perifusion procedure

Male Wistar rats (240–260 g) were anesthetized by an ip injection of pentobarbital (30 mg/kg BW). To obtain an amount of calcitonin measurable by RIA, two thyroid glands were minced with a tissue chopper (McIllwain Tissue-Chopper, Gomshall, UK) and pooled in a perifusion chamber. The perifusion system was previously described (32). Briefly, minced tissue was layered on a cellulose filter at the bottom of a 2-ml disposable syringe (Terumo, Leuven, Belgium) filled with perifusion medium maintained at 37 C in a water bath. The inlet tubing was routed from medium flasks through a peristaltic pump and fixed to a 21-gauge steel needle, which perforated the rubber gasket of the syringe plunger. The outlet tubing was attached to the tip of the barrel and connected to a fraction collector. The plunger was inserted, and the medium volume in the perifusion chamber was adjusted to 0.5 ml. The perifusion medium was a mixture of DMEM-Ham's F-12 medium in Hanks' salts (1:1) slightly modified (0.5 mM calcium) containing 0.5% BSA and aprotinin (200 kallikrein inhibitor units/ml), saturated with a 95% O_2-5% CO_2 mixture, and maintained at pH 7.4. Perifusion was carried out at a flow rate of 300 μl/min, and the effluent was collected at 4 C every 5 min and stored at −20 C until assayed. A stabilization period of 120 min with low calcium (0.5 mM) preceded the experimental phase. The quality of tissue preparations in each perifusion chamber was checked by a 5-mM calcium stimulation for 15 min at the end of the experiments. Calcitonin secretion during the last 15 min of the stabilization period was considered the basal secretory rate of the hormone.

Data analysis and statistics

Measurements were made in either duplicate (binding) or triplicate (calcitonin and cAMP) in each experiment, and data are expressed as the mean ± SEM. Statistically significant differences were determined by unpaired Student's *t* test. Linear regression was calculated by least squares analysis. Receptor binding data were analyzed using a version of the Ligand program (release 2.0, 1990, Biosoft, Cambridge, UK). IC_{50} values (concentrations causing 50% inhibition of maximum binding), ED_{50} values, and Hill coefficients were deduced by fitting the experimental data according to the Hill equation. As ^{125}I-labeled peptide concentrations used for competition curves (~30 pM) were 20% or less of their K_d values, the IC_{50} values of peptides approximated their inhibition constants. For each perifusion, data were expressed as a percentage of the peak value obtained at 5 mM calcium, which was set at 100%.

Results

Binding experiments

Receptor-peptide affinities were estimated on CA-77 membranes by competitive binding of [^{125}I]tGLP-1 and [^{125}I]glucagon with tGLP-1, OXM, and glucagon, respectively. Competitive curves are shown in Fig. 1. The specific binding of [^{125}I]tGLP-1 was 8.1 ± 1.2% (n = 8) of the total radioactivity, using a membrane protein concentration of

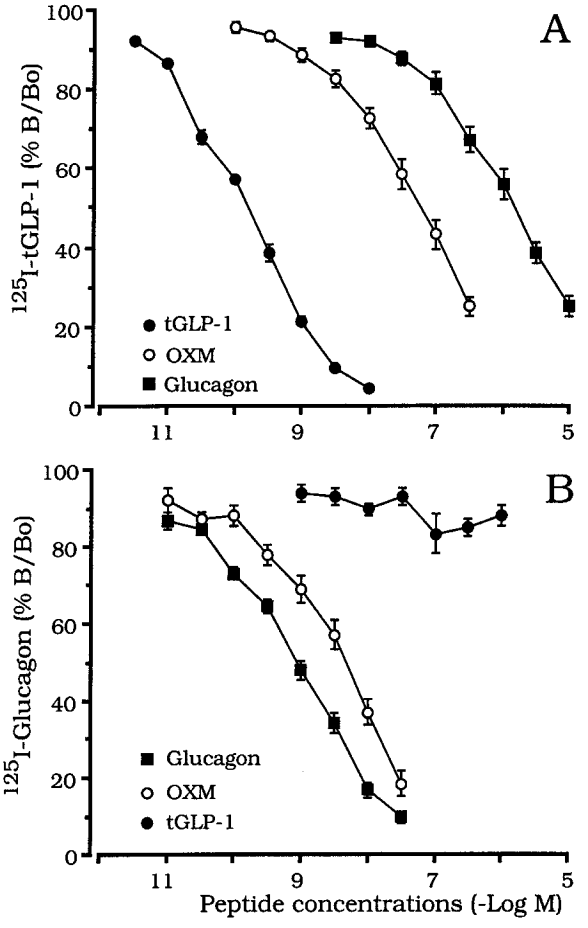

FIG. 1. Competitive inhibition of [^{125}I]tGLP-1 (A) and [^{125}I]glucagon (B) binding to CA-77 membranes by unlabeled tGLP-1 (●), OXM (○), and glucagon (■). Specific binding was expressed as the percentage of maximal specific binding measured with the tracer alone. Each *point* is the mean ± SEM of at least five different experiments, performed in duplicate.

70 ± 8 μg/ml; it was 1.6 ± 0.5% (n = 6) for [^{125}I]glucagon, with a protein concentration of 108 ± 14 μg/ml. The affinities of ^{125}I-labeled peptides for the receptors and the receptor densities determined from Scatchard analyses (Fig. 2) are listed in Table 1. Competitive inhibitions of labeled peptide binding by homologous unlabeled peptides were essentially complete over 2 orders of magnitude, and the best fit was obtained with a one-site model. Hill coefficient values approaching unity indicate a homogeneous population of binding sites for these peptides. The numbers of glucagon- and tGLP-1-binding sites on CA-77 cells, deduced from Scatchard analyses and membrane protein determinations (40 ± 6 μg/10^6 cells; n = 6), were close to 3800 and 8000/cell, respectively.

The rank order of potency of the peptides to inhibit [^{125}I]tGLP-1 binding was as follows (IC_{50}, nanomolar concentrations): tGLP-1 (0.33 ± 0.04; n = 5) > OXM (87 ± 5; n = 4) > glucagon (1540 ± 200; n = 5; Fig. 1A). When using [^{125}I]glucagon, the rank order was (IC_{50}, nanomolar concentrations): glucagon (2.3 ± 0.3; n = 6) > OXM (8 ± 2; n = 5), and no competition with tGLP-1 up to 1 μM was observed (Fig. 1B).

Calcitonin release by CA-77 cells

Calcitonin secretion by CA-77 cells was linearly related to the calcium concentration from 1–4 mM (r = 0.992; $P < 0.001$; n = 9) and reached a maximal value beyond 4 mM calcium (data not shown). At 1, 4, and 5 mM calcium, the calcitonin secretion rates were 157 ± 24, 913 ± 134, and 950 ± 114 pg/10^6 cells·2 h (n = 9), respectively. The calculated half-maximal increase (ED_{50}) was obtained at a calcium concentration of 2.3 mM. In subsequent experiments, a calcium concentration of 2 mM was selected.

As shown in Fig. 3A, tGLP-1 and glucagon dose-dependently stimulated calcitonin secretion by CA-77 cells. The basal calcitonin release was 323 ± 31 pg/10^6 cells·2 h (n = 12). tGLP-1 and glucagon increased calcitonin release by 170 ± 10% (n = 7) and 312 ± 32% (n = 5) over basal values at concentrations of 10 nM and 10 μM, respectively. When the dose-response curves for tGLP-1 were essentially complete over 2 orders of magnitude, reaching a plateau of secretion at 10 nM, those for glucagon spread over 4 orders of magnitude, suggesting interactions of glucagon with receptors other than its own receptor. The half-maximal calcitonin responses (ED_{50}) calculated from Hill's equation for both peptides are reported in Table 1.

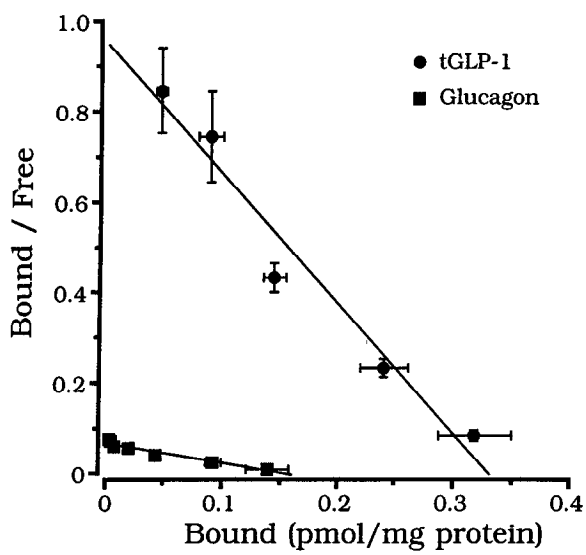

FIG. 2. Scatchard analyses of the tGLP-1 (●) and glucagon (■) displacement curves portrayed in Fig. 1. *Data points* are the mean ± SEM of the number of experiments presented in Table 1.

Cellular cAMP production

tGLP-1 and glucagon stimulated cAMP production by CA-77 cells in a concentration-dependent manner (Fig. 3B). Basal production was 147 ± 14 pmol/10^6 cells·20 min (n = 22), and production rates were not significantly different ($P > 0.05$) after maximal stimulation by tGLP-1 (974 ± 77 pmol/10^6 cells · 20 min; n = 11) and glucagon (901 ± 83 pmol/10^6 cells · 20 min; n = 11). No additivity of the glucagon (1 μM) and tGLP-1 (10 nM) effects was observed on cAMP production (data not shown). As observed for calcitonin secretion, the shape of the dose-response curve was different for tGLP-1 and glucagon. The ED_{50} of tGLP-1 for cAMP production was 1 order of magnitude lower than that of glucagon (Table 1). The Hill coefficient for tGLP-1 was close to 1 (n = 0.936), whereas that of glucagon was 0.519.

Effect of exendin-(9–39) amide on glucagon-stimulated cAMP production and calcitonin release by CA-77 cells

The shape of the dose-response curves for cAMP production and calcitonin release obtained upon stimulation by glucagon revealed the complexity of its action on CA-77 cells. To test the hypothesis that part of the glucagon effect was due to its interaction with tGLP-1 receptors, as depicted in Fig. 1, glucagon-stimulated cAMP production and calcitonin release were determined in the presence or absence of the specific tGLP-1 receptor antagonist, exendin-(9–39) amide (33). This antagonist displays no biological effect on its own, but completely inhibits cAMP production induced by tGLP-1 when added at a 100- to 300-fold excess (33, 34). On CA-77 cells, the increase in cAMP production over basal, induced by 10 nM tGLP-1 (1070 ± 23 pmol/10^6 cells·20 min; n = 6) was decreased by 88.3 ± 2.2% in the presence of a 100-fold excess of exendin-(9–39) amide (126 ± 15 pmol/10^6 cells·20 min; n = 6). As glucagon was 1540-fold less potent than tGLP-1 for tGLP-1 receptor (Fig. 1A), exendin-(9–39) amide and glucagon were added at equimolar concentrations in the subsequent experiments.

As shown in Fig. 4A, the dose-response curve for cAMP production obtained with glucagon in the presence of exendin-(9–39) amide was different from that obtained without exendin-(9–39) amide at glucagon concentrations over 10 nM. The curve in the presence of exendin-(9–39) amide displayed a sigmoidal shape, over 2 orders of magnitude, with a maximal effect close to 10 nM glucagon, an ED_{50} of 0.40 ± 0.10 nM, and a Hill's coefficient of 0.971 ± 0.13 (n = 3). Glucagon-induced calcitonin release in the presence of equimolar concentrations of exendin-(9–39) amide (Fig. 4B) was modified at glucagon concentrations greater than 10 nM, with a rightward shift of the curve by 1 order of magnitude. Glucagon-induced calcitonin release, at concentrations

TABLE 1. Binding and biological constants of receptor sites for tGLP-1 and glucagon in the CA-77 cell line

Peptides	Binding			Bioactivities			
	K_d (nM)	B_{max} (pmol/mg protein)	Hill coefficient	Calcitonin release, ED_{50} (nM)	Hill coefficient	cAMP production, ED_{50} (nM)	Hill coefficient
tGLP-1	0.33 ± 0.05 (5)	0.33 ± 0.04 (5)	0.923 ± 0.04 (5)	0.17 ± 0.07 (7)	0.961 ± 0.05 (7)	0.34 ± 0.05 (11)	0.936 ± 0.06 (11)
Glucagon	2.3 ± 0.3 (6)	0.16 ± 0.02 (6)	0.973 ± 0.01 (6)	54 ± 13 (5)	0.488 ± 0.07 (5)	3.7 ± 0.7 (11)	0.519 ± 0.03 (11)

Values are the mean ± SEM of tests performed in triplicate (bioactivities) or in duplicate (binding). ED_{50} values were calculated from Hill's equation. Dissociation constant (K_d) and receptor density (B_{max}) are deduced from Scatchard analysis of inhibition curves using monoiodinated and unlabeled homologous peptides (see Fig. 2). The number of experiments is shown in *parentheses*.

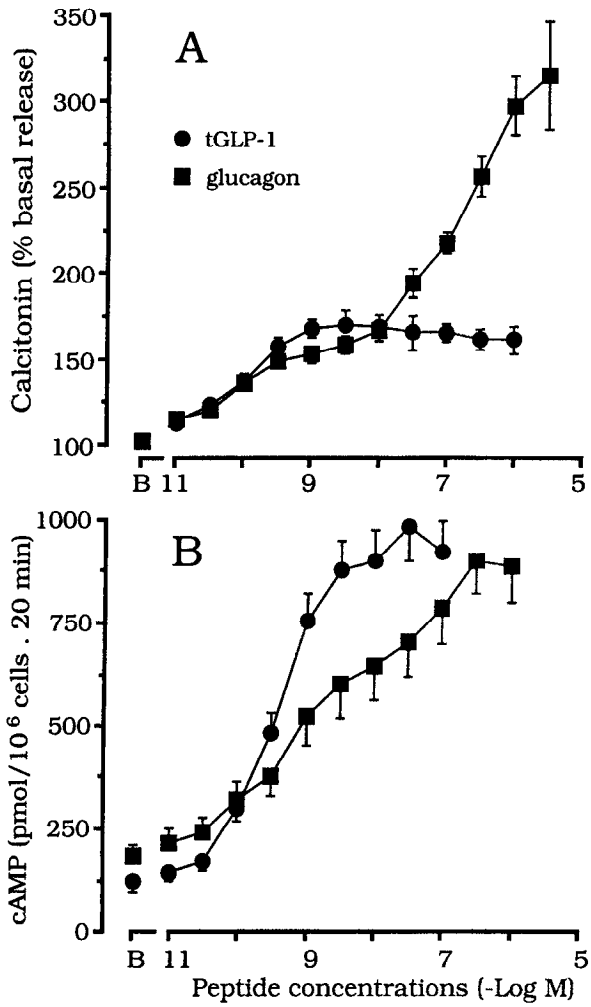

FIG. 3. Effects of glucagon (■) and tGLP-1 (●) on calcitonin secretion (A) and intracellular cAMP production (B) by CA-77 cells. Calcitonin release and cAMP formation were measured as described in *Materials and Methods*. Calcitonin was expressed as a percentage of basal release. Values are the mean ± SEM of at least five different experiments, performed in triplicate.

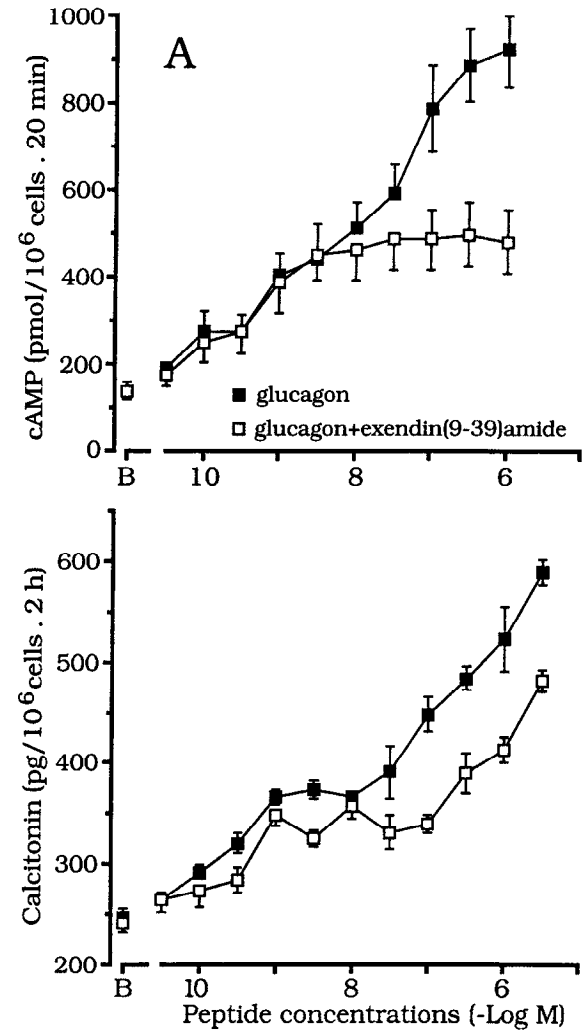

FIG. 4. Effect of exendin-(9–39) amide on glucagon-induced calcitonin release (A) and glucagon-induced cAMP production (B) by CA-77 cells. For each *point*, exendin-(9–39) amide was added at concentration equimolar to that of glucagon. Values are the mean ± SEM of three tests, performed in triplicate.

greater than 100 nM, was not correlated with a further increase in cAMP production.

Perifusion studies

As the expression of glucagon and tGLP-1 receptor sites might be related to a tumoral feature of the CA-77 cell line, the effects of glucagon and tGLP-1 were compared on isolated rat thyroid glands perifused *in vitro*.

Peptides were added to the perifusion medium during a 30-min period, 30 min after the calcium medium was increased from 0.5 to 1 mM (Fig. 5A) or to 3 mM (Fig. 5, B and C). The basal calcitonin secretory rate (at 0.5 mM calcium) and the maximal secretory rate (at 5 mM calcium) were 26.7 ± 1.2 and 178 ± 18 pg/min · two thyroids (n = 36), respectively. Calcitonin release was unaffected by an increase in the calcium concentration from 0.5 to 1 mM and by the addition of 10 nM glucagon or tGLP-1 (Fig. 5A). When the calcium concentration was increased from 0.5 to 3 mM (Fig. 5, B and C),

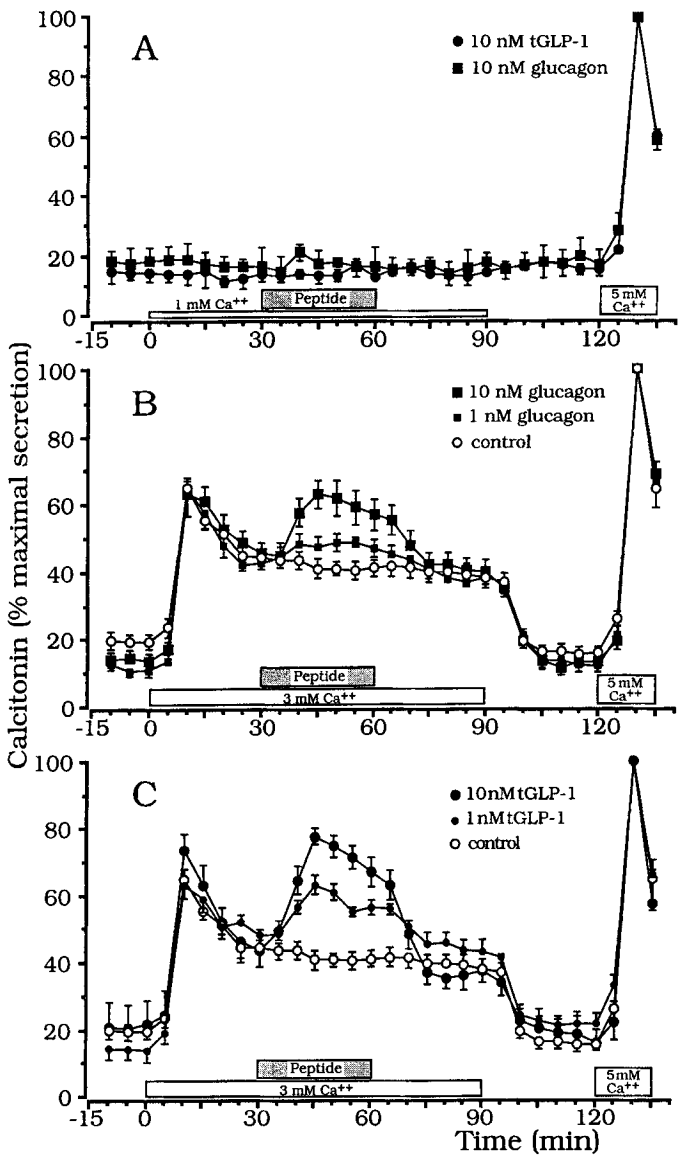

FIG. 5. Effect of glucagon (■) or tGLP-1 (●) on calcitonin secretion from perifused rat thyroid in the presence of 1 mM calcium (A) and 3 mM calcium (B and C). A stabilization period of 120 min at 0.5 mM calcium preceded the stimulation phase of 90 min at either 1 or 3 mM calcium and was followed by a 30-min period at 0.5 mM calcium. Perifusion terminated with of a 15-min period at 5 mM calcium for viability control of each preparation. Results are the mean ± SEM of four to eight experiments and are expressed as a percentage of the maximal secretion obtained at 5 mM calcium for each perifusion.

calcitonin release rose rapidly to peak at 10 min (~65% of the maximal secretion value), followed by a progressive decline to a sustained plateau that was about 40% of the maximal secretion value. A rapid decrease in calcitonin release occurred after reexposure of the thyroids to 0.5 mM calcium medium. In association with 3 mM calcium, glucagon (Fig. 5B) and tGLP-1 (Fig. 5C) elicited an additional and dose-dependent sustained release of calcitonin.

Discussion

The present data demonstrate the existence of specific receptors for glucagon and its structurally related peptide tGLP-1 on the C cell line CA-77, derived from a rat medullary thyroid carcinoma. The binding characteristics of glucagon receptors on CA-77 plasma membranes are similar to those described on liver membranes (35) with a potency for oxyntomodulin 5-fold less than that of glucagon, as previously reported (36), and no cross-reactivity with tGLP-1 up to 1 μM. The binding characteristics of tGLP-1 receptors on CA-77 membranes agree with those found in other murine cell lines, RINm5F (26, 37) and RIN T3 (21, 26), originating from the endocrine pancreas and in another cultured medullary carcinoma of rat thyroid, rMTC 6–23 (24). As previously reported in RIN T3 cells (21), the affinities of oxyntomodulin and glucagon for tGLP-1 receptors are 2 and 3 orders of magnitude lower than that of tGLP-1, respectively.

Both receptors are positively coupled to the adenylate cyclase-cAMP system, but unlike tGLP-1, the dose-response curve for glucagon covers a range of concentrations (0.03–316 nM), which does not correlate with that used in binding experiments (0.03–10 nM). These data, together with a Hill coefficient less than 1 (n = 0.519), suggest interactions of glucagon with receptors, other than its own receptor, coupled to the adenylate cyclase system. As glucagon inhibits the binding of [^{125}I]tGLP-1 to the tGLP-1 receptor at concentrations higher than 10 nM, it is likely that glucagon stimulates cAMP production through interaction with this receptor. This hypothesis is confirmed by the experiments with exendin-(9–39) amide, a specific receptor antagonist of tGLP-1 (33, 34). Indeed, the antagonist had no effect on glucagon-induced stimulation of cAMP production at glucagon concentrations from 0.03–10 nM, but abolished a further increase in cAMP production at higher glucagon concentrations. Glucagon and tGLP-1 might act on the same cAMP pool in CA-77 cells because no additive action was observed at a maximally effective concentration of either peptide. Furthermore, the experiments performed with exendin-(9–39) amide suggest that unlike tGLP-1, glucagon is not able to fully stimulate the hormone-sensitive adenylate cyclase system through its own receptor.

The present results confirm that the CA-77 cell line releases calcitonin in response to a rise in the extracellular calcium concentration and that the response is qualitatively similar to that reported previously with the same C cell line (27) and by several investigators using transplantable rat medullary thyroid carcinoma (9) or another C cell line, rMTC 6–23 (10). The present investigation shows that glucagon and tGLP-1 stimulate calcitonin secretion in CA-77 cells through several pathways depending on the concentrations used. It is likely that at low concentrations, glucagon interacts with its own receptor and induces calcitonin secretion via a cAMP-dependent pathway. At high glucagon concentrations, the mechanism of action seems more complex and may involve an interaction with tGLP-1 receptor, and an activation of an additional intracellular messenger, independent from the cAMP pathway through interaction with an undefined receptor.

CA-77 cells provide an example of a cell line that clearly expresses functional glucagon and tGLP-1 receptors. Indeed, in the other rat C cell line (rMTC 6–23) in which tGLP-1 receptors were identified (24), glucagon failed to stimulate adenylate cyclase (24) or increase cAMP production and

calcitonin release at concentrations higher than 0.1 μM (10, 11). However, in another report using the same cell line, glucagon evoked cAMP and calcitonin responses with half-maximal effective concentrations of about 10 and 50 nM, respectively (38). The presence of glucagon and tGLP-1 receptors in CA-77 cells, and presumably in rMTC 6–23 cells, probably reflects their expression in normal thyroid C cells. Indeed, glucagon and tGLP-1 stimulate calcitonin release by rat thyroid glands perifused *in vitro*. This process occurs at nanomolar peptide concentrations, in agreement with the affinity for their own receptors. Furthermore, as the affinity of glucagon to tGLP-1 receptor is ~1500-fold less than that of tGLP-1 (Fig. 1A), the reported effects of nanomolar concentrations of glucagon on calcitonin secretion are not mediated through interaction with the tGLP-1 receptor. In perifusion experiments, the calcitonin responses to glucagon and tGLP-1 stimulations were calcium dependent. Indeed, at a low calcium concentration (1 mM), both peptides failed to stimulate calcitonin secretion, whereas at a high calcium concentration (3 mM), which stimulated calcitonin release *per se*, they further increased calcitonin secretion. The potentiation of calcium-induced calcitonin secretion by glucagon in rat thyroids confirms previously reported synergistic secretagogue interactions between glucagon and calcium on primary cell cultures from transplantable rat medullary thyroid carcinoma (9). However, they are at variance with the additive effects of glucagon and calcium on calcitonin secretion reported in the rMTC 6–23 cell line (11). The reason for this discrepancy is unclear and might reflect intrinsic differences between normal C cells and C cell lines.

The present data are in agreement with the concept, expressed 25 yr ago and referred to as the gastroenteroparafollicular axis (13, 39), that peptides are released from the gut during the absorption of calcium to alert C cells of an impending influx of calcium so that they can respond early enough to prevent subsequent hypercalcemia. It has been found that small amounts of calcium administered into the jejunum, although insufficient to increase systemic plasma calcium, stimulate calcitonin secretion, suggesting that a signal arose from the intestinal tract (40). Among several humoral factors, glucagon-like immunoreactive peptide, or enteroglucagon, has been proposed because it meets two necessary conditions: 1) enteroglucagon is increased after intraduodenal administration of calcium (13); and 2) nanomolar concentrations of enteroglucagon perfused through the thyroidal artery stimulate the calcitonin secretion rate (12). However, enteroglucagon corresponds to a set of circulating glucagon-containing peptides (mainly glicentin and oxyntomodulin) coproduced (15, 16) and coreleased (17–19) with tGLP-1 by the L cells of the intestine. Specific oxyntomodulin receptors have not yet been identified, but the molecule has been shown to interact with glucagon and tGLP-1 receptors (21, 36) with potencies 5- and 100-fold less than those of the homologous peptide, respectively. Consequently, it is unlikely that oxyntomodulin might be a candidate to potentiate *in vivo* calcium-induced calcitonin secretion. Because tGLP-1 displays on Sephadex G-50 columns, mainly used for enteroglucagon purification, a coefficient of distribution near that of oxyntomodulin (17), it might represent the active factor that in the enteroglucagon preparations stimulated calcitonin secretion (12).

Beyond the well documented incretin effect of tGLP-1 (41), it has been proposed as an enterogastrone, responsible for the inhibition of gastropancreatic secretion in the presence of unabsorbed nutrients in the ileum (42). From the present investigation, it is tempting to speculate that tGLP-1 provides a safeguard against major changes in calcemia resulting from the ingestion of a large amount of calcium through a gastroentero-thyroid C cell axis.

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