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Different pharmacological characteristics in L_6 and C_2C_{12} muscle cells and intact rat skeletal muscle for amylin, CGRP and calcitonin

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1 We compared the ability of rat amylin, rat calcitonin gene-related peptide (CGRP) and rat and salmon calcitonins to elevate cyclic AMP levels and to inhibit [U-^{14}C]-glucose incorporation into glycogen in insulin-stimulated intact rat soleus muscle and in two cell lines derived from rodent skeletal muscle, L_6 and C_2C_{12}

2 In intact soleus muscle, both amylin (EC_{50}s of 0.7–6.1 nM) and salmon calcitonin (EC_{50}s of 0.5–1.4 nM) were more potent than CGRP (EC_{50}s of 5.6–15.8 nM) and were much more potent than rat calcitonin (EC_{50}s of 50–137 nM) at stimulating cyclic AMP production, activating glycogen phosphorylase and inhibiting insulin-stimulated [^{14}C]-glycogen formation.

3 In contrast, in both L_6 and C_2C_{12} cells, CGRP (EC_{50}s of 0.042–0.12 nM) stimulated cyclic AMP formation and inhibited insulin-stimulated [U-^{14}C]-glucose incorporation into glycogen approximately 1000 times more potently than amylin (EC_{50}s 34–240 nM), while salmon calcitonin was without measurable effect.

4 There was a correlation between elevation of cyclic AMP and inhibition of insulin-stimulated [U-^{14}C]-glucose incorporation into glycogen evoked by these peptides in both intact muscle ($r^2=0.69$, $P<0.0004$) and muscle cell lines ($r^2=0.96$, $P<0.0001$).

5 In conclusion, the effects of amylin, CGRP, and calcitonin on soleus muscle glycogen metabolism appear to be mediated by adenylyl cyclase-coupled receptors which show a pharmacological profile similar to high affinity amylin binding sites that have been previously reported in rat brain. In contrast, the effects of amylin and CGRP in L_6 and C_2C_{12} rodent muscle cell lines appear to be mediated by adenylyl cyclase-coupled receptors that behave like CGRP receptors.

Keywords: Amylin; CGRP; calcitonin; peptide receptors; skeletal muscle; L_6; glucose metabolism; cyclic AMP

Introduction

Amylin, a 37 amino acid peptide that is released from pancreatic β-cells, shares approximately 50% amino acid sequence identity with calcitonin gene-related peptide (CGRP), a 37 amino acid neuropeptide that is widely distributed in peripheral nerves (Cooper *et al.*, 1988; Rink *et al.*, 1993; Pittner *et al.*, 1994; Cooper, 1994). Both amylin and CGRP can inhibit insulin-stimulated incorporation of [U-^{14}C]-glucose into glycogen in skeletal muscle (Leighton & Cooper, 1988; Deems *et al.*, 1991a; Leighton *et al.*, 1989; Young *et al.*, 1992; Rossetti *et al.*, 1993; Beaumont *et al.*, 1995b). Amylin increases glycogen phosphorylase activity and reduces glycogen synthase activity in skeletal muscle (Deems *et al.*, 1991b; Young *et al.*, 1991; Lawrence & Zhang, 1994; Pittner *et al.*, 1995a), promoting glycogenolysis and lactate release (Leighton & Cooper, 1988; Pittner *et al.*, 1995a). These effects appear to be associated with activation of adenylyl cyclase (Pittner *et al.*, 1995a); although some reports suggest that additional or alternative signalling mechanisms may be involved (Lawrence & Zhang, 1994; Deems *et al.*, 1991a; Kreutter *et al.*, 1993).

In intact skeletal muscle, recent studies with selective antagonists indicate that amylin and CGRP regulate glycogen metabolism by activation of a common population of receptors (Beaumont *et al.*, 1995b) that appears pharmacologically similar to amylin receptors in rat brain (Beaumont *et al.*, 1993; Sexton *et al.*, 1994; Van Rossum *et al.*, 1994); i.e. amylin is at least as potent as CGRP, and salmon calcitonin is most potent in effects on glycogen metabolism (Beaumont *et al.*, 1995b; Young *et al.*, 1995). This is in sharp contrast to the pattern of interaction of these ligands with CGRP receptors, which bind CGRP best, bind amylin with ~100 fold lower affinity, and bind salmon calcitonin very weakly, if at all (Yamaguchi *et al.*, 1988; Poyner *et al.*, 1992).

Cell models offer some advantages over tissues and whole animals in the study of cellular functions. Effects of amylin and CGRP have been studied in two skeletal muscle cell lines, rat L_6 cells and mouse C_2C_{12} cells. Rat L_6 cells appear to express CGRP receptors coupled to adenylyl cyclase activity (Kreutter *et al.*, 1989, 1993; Zhu *et al.*, 1991; Poyner *et al.*, 1992); these receptors are activated by amylin with low potency relative to CGRP (Zhu *et al.*, 1991; Poyner *et al.*, 1992). However, potent effects of amylin have also been reported in C_2C_{12} cells (Sheriff *et al.*, 1992).

In order to characterize the receptors involved, and to assess whether muscle cell lines would be useful models for amylin action in intact skeletal muscle, we compared the relative potencies of rat amylin, rat CGRP, rat calcitonin and salmon calcitonin in activating adenylyl cyclase and inhibiting glycogen synthesis in intact skeletal muscle and L_6 and C_2C_{12} muscle cell lines.

Methods

Soleus muscle dissection and incubation

Soleus muscle strips were obtained from male Sprague-Dawley Rats (170–200g), as described (Young *et al.*, 1992). Muscle strips were incubated for 20–30 min in physiological saline at

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room temperature and then in Krebs-Ringer bicarbonate (KRB) buffer containing (mM): NaCl 118.5, KCl 5.94, $CaCl_2$ 2.54, $MgSO_4$ 1.19, KH_2PO_4 1.19, $NaHCO_3$ 25 and glucose 5.5 at pH 7.3. Incubations were either performed in 20 ml polyethylene scintillation vials with 2 ml of KRB buffer containing 0.1% fatty acid-free bovine serum albumin (BSA) and continuously gassed with Carbogen (95% O_2:5% CO_2) at 37°C, or in 6 well culture plates in 2 ml of buffer/well. Culture plates were placed in a modular incubation chamber (Billups-Rothenberg, Del Mar, CA, U.S.A.) which was flushed with Carbogen for 30 s before placing on an orbital rotator inside a 37°C incubator. This chamber was re-flushed each time the chamber was opened.

L_6 and C_2C_{12} culture

L_6 cells were seeded at 5×10^4 cells/well in 6-well plates in Dulbecco's modification of Eagle's medium (DMEM) supplemented with glutamine, penicillin, streptomycin and 10% foetal calf serum (FCS). Once cells approached confluency the serum content was reduced to 2%. After reaching confluency, cells were maintained for at least three days before use to ensure fusion into myotubes.

C_2C_{12} cells were plated at 5×10^4 cells/well in 6-well plates in DMEM supplemented with glutamine, penicillin, streptomycin, 20% FCS and 0.5% chick embryo extract (Gibco BRL) and were grown to 70% confluency at 37°C in humidified atmosphere containing 5% CO_2. Fusion of cells was induced by replacing FCS and chick embryo extract with 10% horse serum. Cells fused into multinucleated myotubes after a further 7–10 days in culture (Sheriff *et al.*, 1992). Medium was changed daily to avoid detachment of cells.

Cyclic AMP determination

Soleus muscles were incubated for 10 min as described above in KRB buffer containing 0.1% BSA after which muscles were removed rapidly, blotted dry, trimmed of tendons, frozen in liquid N_2 and weighed; 0.5 ml of ice-cold 5% TCA was then added to each muscle piece and sonicated as described below. Samples were neutralized by the addition of 0.2 ml of 0.8 M Trizma and spun briefly to separate particulate material. Adenosine 3′: 5′-cyclic monophosphate (cyclic AMP) in the supernatant was determined by radioimmunoassay. Results are expressed as pmol cyclic AMP mg^{-1} wt. of tissue. Cyclic AMP levels remain elevated for 20 min (Pittner *et al.*, 1995a) and a 10 min incubation was chosen as this was also an optimal time for measuring glycogen phosphorylase.

L_6 and C_2C_{12} cells were incubated for 20 min in PBS buffer containing 0.1% BSA, pH 7.4 at 37°C in a humidified air incubator. Cells were then incubated for 10 min in the same buffer containing 0.5 mM isomethylbutylxanthine (IBMX), followed by an additional 10–15 min in IBMX buffer containing hormones as indicated. The medium was then removed and 0.5 ml of ice-cold 5% TCA was added and plates were kept on ice for 15 min. Following addition of 0.2 ml of 0.8 M Trizma, the cells were scraped and sonicated for 10 s at setting 10 on a MSE Soniprep 150 fitted with an exponential microtip and frozen at −70°C. Cyclic AMP was determined by radioimmunoassay. Results are expressed as pmol cyclic AMP produced/well.

Glycogen phosphorylase activity

Glycogen phosphorylase activity was determined in muscle pieces incubated as described for cyclic AMP studies, from the rate of the reverse reaction, glucose 1-phosphate incorporation into glycogen (Hue *et al.*, 1975; Stalmans & Hers, 1975), as measured by the release of inorganic phosphate (LeBel *et al.*, 1978). This assay excludes the measurement of glycogen phosphorylase b, which depends upon the additional presence of AMP. Muscle pieces were sonicated as described above in 1 ml of ice cold buffer containing 100 mM NaF, 20 mM ethylenediaminetetraacetic acid (EDTA), 50 mM Trizma and 0.5% glycogen (mussel) at pH 6.5. Each sample (0.2 ml) was added to incubation buffer (0.2 ml) containing 300 mM NaF, 50 mM glucose 1-phosphate, 10 mM caffeine and 2% glycogen at pH 6.1 for determination of glycogen phosphorylase *a* activity. Incubations for 20 min at 30°C were terminated by the addition of 0.1 ml of ice-cold TCA (20%), and inorganic phosphate was measured by the ammonium molybdate method (LeBel *et al.*, 1978). Results are expressed as pmoles P_i produced min^{-1} mg^{-1} wet wt of tissue.

[U-^{14}C]-glucose incorporation into glycogen

Incorporation of glucose into glycogen in rat soleus muscle was determined as described in detail previously (Young *et al.*, 1992). L_6 and C_2C_{12} cells were incubated for 5 h in serum-free DMEM, as for glucose uptake studies, except that 5.5 mM glucose was added. Cells were then washed with PBS containing 0.1% BSA and incubated for 60 min in PBS buffer containing 0.1% BSA, hormones and peptides as indicated and 5.5 mM [U-^{14}C]-glucose (0.5 μCi ml^{-1}) at 37°C in a humidified air incubator. Plates were then placed on ice and washed 3 times with ice-cold PBS buffer and reactions terminated by the addition of 1 ml of 0.1 M NaOH. Digested cells were then transferred to 5 ml polypropylene tubes to which 200 μl of a stock 40 mg ml^{-1} glycogen solution was added. Samples were placed at 75°C for an additional 15 min before being cooled on ice. Ice-cold 100% ethanol (2.4 ml) was added and samples were vortexed and placed on ice for 30 min before being centrifuged. The supernatant was aspirated and the pellet was washed twice with 2 ml of ice-cold ethanol, and then dried prior to being reconstituted in 1 ml of water for determination of radioactivity. Results are expressed as nmol glucose incorporated h^{-1} per well.

We wish to emphasize that this assay measures a rate of labelling of glycogen that is normalized to glucosyl units using the specific activity of the tracer in glucose. We have discussed elsewhere (Young *et al.*, 1992) that this measure reflects events in muscle glycogen metabolism, but does not necessarily quantitate to net glycogen accumulation, since rates of labelling are positive even during net glycogen breakdown.

Materials

Rat amylin, rat CGRP, rat and salmon calcitonin were from Bachem California (Torrance, CA, U.S.A.). They were dissolved in deionized water in polypropylene tubes at 200 μM and stored at −70°C. Recombinant human insulin (Humulin-R) was from Eli Lilly (Indianapolis, IN, U.S.A.). Other chemicals were of reagent grade from Sigma Co. (St. Louis, MO, U.S.A.) or Fisher Scientific (Atlanta, GA, U.S.A.). D-[U-^{14}C]-glucose, L-[^{3}H]-glucose and [^{3}H]-2-deoxyglucose were from Amersham Corp (Arlington Heights, IL, U.S.A.), and the cyclic AMP radioimmunoassay kit was from Biomedical Technologies Inc (Stoughton, MA, U.S.A.). L_6 (Yaffe, 1968) and C_2C_{12} cells were obtained from ATCC (Rockville, MD, U.S.A.).

Numerical methods

The number of independent experiments and the total number of muscle strips incubated under each assay condition are specified in the relevant figure legends. Results of studies on cultured cells are expressed as means ± s.e.mean from 3–4 independent experiments. Dose-response curves were fitted to a four-parameter logistic equation (Inplot; GraphPAD Software, San Diego, CA, U.S.A.). EC_{50} values are expressed with 95% confidence intervals (95% CL). Statistical analysis was performed using unpaired *t* test where appropriate (Instat; GraphPAD software, San Diego, CA, U.S.A.).

Results

Effects of rat amylin, rat CGRP, rat calcitonin and salmon calcitonin in rat soleus muscle

Cyclic AMP Following 10 min incubation in the absence of phosphodiesterase inhibitors, rat amylin and salmon calcitonin increased cyclic AMP in rat soleus muscle (Figure 1a), with EC_{50}s of 0.7 nM and 1.4 nM, respectively (Table 1). Rat CGRP was approximately 10 fold less potent than rat amylin and rat calcitonin was more than 100 fold less potent.

Glycogen phosphorylase Rat amylin and salmon calcitonin also potently stimulated increases in glycogen phosphorylase activity in rat isolated soleus muscle, with EC_{50}s of 2.2 nM and 1.1 nM, respectively (Figure 1b). Rat CGRP was 2.5 fold less potent than rat amylin, and rat calcitonin was 23 fold less potent than rat amylin at increasing glycogen phosphorylase activity (Table 1).

[^{14}C]-glycogen formation The relative potency of rat amylin, rat CGRP and salmon calcitonin at inhibiting insulin-stimulated [^{14}C]-glycogen formation has been reported previously (Beaumont *et al.*, 1995b; Young *et al.*, 1995), and is shown for comparison in Table 1. Salmon calcitonin was approximately 14 fold more potent than rat amylin. Rat CGRP was 2.6 fold less potent than rat amylin, while rat calcitonin was 23 fold less potent than rat amylin.

Effects of amylin, CGRP and calcitonin in rodent skeletal muscle cell lines

Rat L_6 cells In marked contrast to the order of potencies observed in intact soleus muscle, rat amylin was ~2000 fold less potent than rat CGRP in stimulating cyclic AMP production in rat L_6 cells with EC_{50}s of 240 nM and 0.12 nM, respectively (Figure 2; Table 1). Rat amylin was 340 fold less potent at stimulating cyclic AMP synthesis in rat L_6 cells than in rat soleus muscle; CGRP was ~70 fold more potent for this action in L_6 cells than in soleus muscle.

As in intact soleus muscle ($r^2=0.69$, $P<0.0004$), the ability of ligands to inhibit [^{14}C]-glycogen formation in L_6 cells paralleled their ability to stimulate cyclic AMP synthesis (Figure 2; $r^2=0.96$, $P<0.0001$). In marked contrast to the pattern observed in intact soleus muscle, rat CGRP was more potent than rat amylin in L_6 cells; at a concentration of 100 nM, salmon calcitonin had no effect on either cyclic AMP production (98±28% of control, $n=4$) or [^{14}C]-glycogen formation (100±9% of control, $n=4$).

Mouse C_2C_{12} cells In C_2C_{12} cells, as in L_6 cells, rat CGRP was considerably more potent than rat amylin in stimulating cyclic AMP synthesis (Figure 3a) and in inhibiting insulin-stimulated [^{14}C]-glycogen formation (Figure 3b). Salmon calcitonin (100 nM) did not measurably alter either cyclic AMP concentration (Figure 3a) or [^{14}C]-glycogen formation in C_2C_{12} cells (Figure 3b).

Thus, in both rat L_6 cells and in mouse C_2C_{12} cells, for both cyclic AMP generation and for the inhibition of [^{14}C]-glycogen formation, the order of potency was CGRP>>rat amylin>>salmon calcitonin. This order differed from that observed in rat soleus muscle.

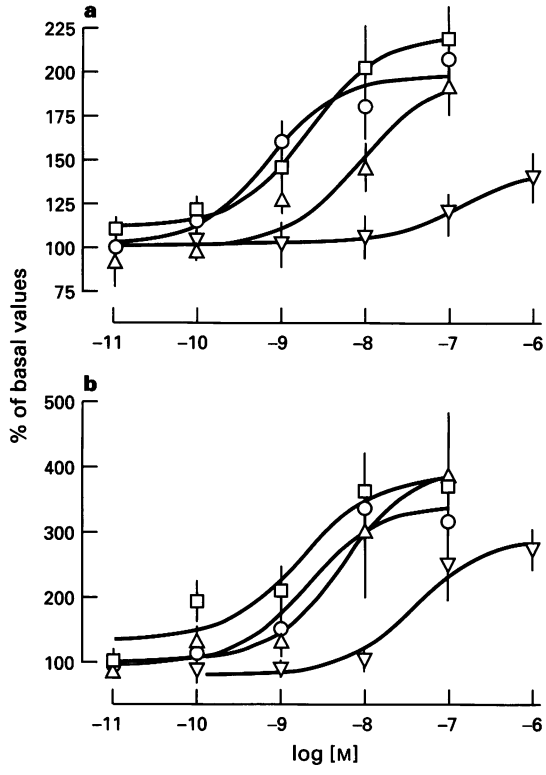

Figure 1 Dose response curves for (a) accumulation of cyclic AMP, (b) activation of glycogen phosphorylase. Results are expressed as % of control values seen in the absence of added hormones. Results are means±s.e.mean of 8–20 muscle strips from 3–6 independent experiments for salmon calcitonin (□) and rat calcitonin (▽), 20–44 muscle strips from 8–11 independent experiments for rat amylin (○) and 8–16 muscle strips from 3–4 independent experiments for rat αCGRP (△). Results for amylin in (a) and (b) are reproduced from (Pittner *et al.*, 1995a). For cyclic AMP, control values were 0.30±0.01, 0.32±0.03, 0.38±0.02 and 0.36±0.01 pmol cyclic AMP mg^{-1} wet wt. for amylin, sCT, rCT and rαCGRP respectively. Control values for glycogen phosphorylase were 378±37, 292±48, 472±50 and 421±49 pmol Pi produced $min^{-1}mg^{-1}$ wet wt. for amylin, sCT, rCT and rαCGRP respectively.

Table 1 EC_{50}s and 95% confidence intervals (95% CL) were calculated from dose-response curves shown in Figures 1 and 2

	Rat amylin		*Rat αCGRP*		*Salmon calcitonin*		*Rat calcitonin*	
	EC_{50} (nM)	95% CL	EC_{50} (nM)	95% CL	EC_{50} (nM)	95% CL	EC_{50} (nM)	95% CL
Soleus								
Cyclic AMP	0.7	0.06–8.8	8.5	0.9–74	1.4	0.45–5.3	131	50–349
Phosphorylase a	2.2	0.6–8.4	5.6	2.4–13.1	1.1	0.3–4.0	50	10.8–232
Glycogen synthesis	6.1	3.1–12.1	15.8	8.0–31.1	0.45	0.26–0.78	137	50.3–376
L6 cells								
Cyclic AMP	240	74.3–745	0.12	0.06–0.25	NE		NT	
Glycogen synthesis	33.8	14.9–75.6	0.042	0.03–0.06	NE		NT	

Data on soleus muscle glycogen synthesis were from previously reported studies (Beaumont *et al.*, 1995a; Young *et al.*, 1995). NE, no effect seen at 100 nM; NT, not tested.

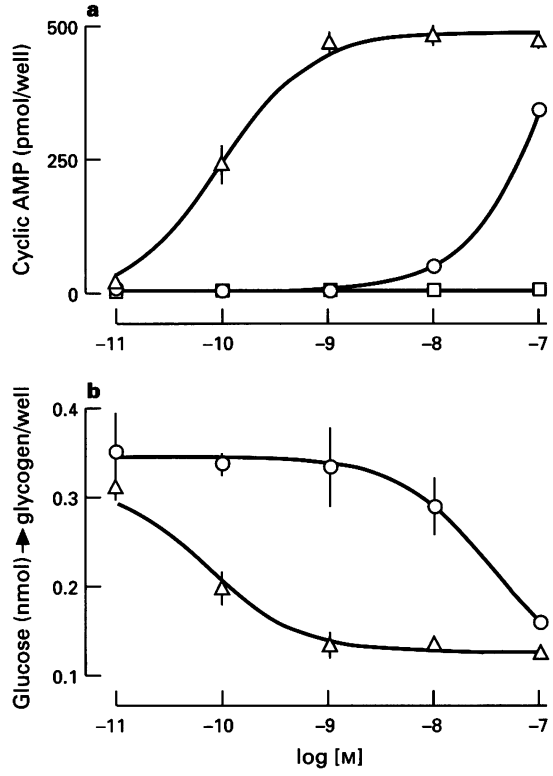

Figure 2 Dose-response curves for (a) accumulation of cyclic AMP and (b) incorporation of [U-^{14}C]-glucose into glycogen in L_6 cells. Effects of rat αCGRP (△), rat amylin (○) and salmon calcitonin (□) were measured in the absence (a) or presence (b) of insulin (10 nM) as described in methods section. Results are mean ± ranges of duplicate samples from a representative experiment.

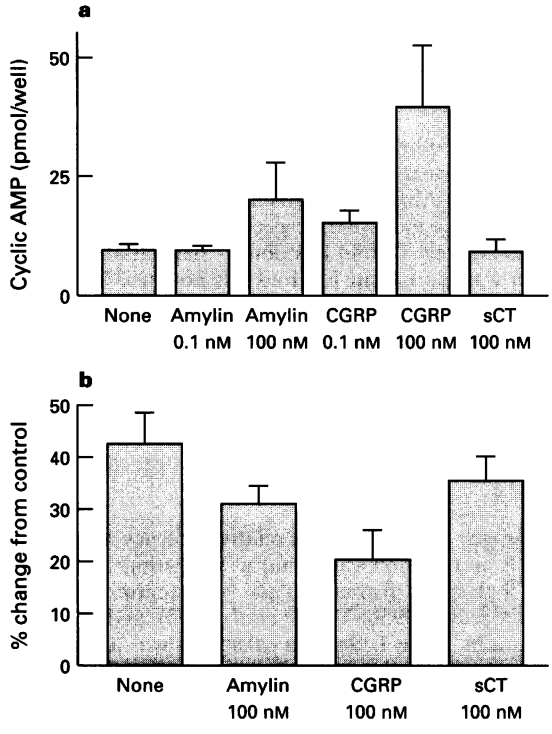

Figure 3 Effects on (a) cyclic AMP production and (b) glycogen synthesis in C_2C_{12} cells. As in Figure 2, effects of hormones on cyclic AMP generation were measured in the absence of insulin (a), while effects on [^{14}C]-glycogen formation were measured in the presence of 10 nM insulin (b). Results are expressed as (a) % change in cyclic AMP accumulation from incubations performed in the absence of hormones (9.2±1.5 pmol cyclic AMP/well); and (b) % change in glycogen synthesis from incubations performed in the presence of insulin (glycogen synthesis 1.12±0.8 nmol glucose incorporated h^{-1} per well, was increased 42±6% by 10 nM insulin). Results are means±s.e.mean from 3-4 independent experiments.

While orders of potency of the tested ligands were similar in mouse C_2C_{12} cells and rat L_6 cells, the cell lines differed in relative responsiveness. Rat CGRP produced only a 4 fold increase in cyclic AMP in C_2C_{12} cells (Figure 3a) compared to a 60 fold increase in L_6 cells. In contrast, adrenaline (1 μM) stimulated cyclic AMP accumulation nearly equally in each cell line, 66 fold (6.4±0.4 to 425±158 pmol cyclic AMP/well) in L_6 cells and 54 fold (9.2±1.5 to 498±120 pmol cyclic AMP/well) in C_2C_{12} cells.

Discussion

In the present study, we compared the ability of rat amylin, rat CGRP, rat calcitonin, and salmon calcitonin to increase cyclic AMP, glycogen phosphorylase activity or inhibit insulin-stimulated [^{14}C]-glycogen formation in intact rat soleus muscle and in the rodent-derived muscle cell lines, C_2C_{12} and L_6.

In rat soleus muscle, rat amylin was approximately equipotent with salmon calcitonin, was ~10 fold more potent than rat CGRP, and was at least 100 fold more potent than rat calcitonin at increasing cyclic AMP. A similar order of potencies (salmon calcitonin ≥ rat amylin > CGRP > > rat calcitonin) was also apparent for the activation of glycogen phosphorylase and the inhibition of [^{14}C]-glycogen formation in soleus muscle. The relative potencies of these peptides for these responses in rat soleus muscle were similar to their reported relative affinities for amylin receptors identified in rat brain (Beaumont *et al.*, 1993). These findings are consistent with recent results suggesting that amylin activates adenylylcyclase coupled receptors in rat soleus muscle (Pittner *et al.*, 1995a) that are pharmacologically similar to high affinity amylin receptors identified in rat brain (Beaumont *et al.*, 1995b) and distinct from CGRP receptors (Yamaguchi *et al.*, 1988; Poyner *et al.*, 1992).

In contrast to the pattern seen in soleus muscle, the order of potency for cyclic AMP generation and inhibition of [^{14}C]-glycogen formation in both L_6 and C_2C_{12} cells was CGRP > > rat amylin > > salmon calcitonin. The 100 to 1000 fold greater potency for CGRP than rat amylin for the actions found here in both L_6 cells and C_2C_{12} cells (Figures 2 and 3) agrees with previous studies reporting the presence in L_6 cells of adenylyl cyclase-coupled receptors with much lower affinity for amylin than for CGRP (Zhu *et al.*, 1991; Poyner *et al.*, 1992; Kreutter *et al.*, 1989; 1993). The order of potency, CGRP > > rat amylin > > salmon calcitonin, concurs with the order of affinity to CGRP receptors, as shown in Table 1, and suggests that CGRP receptors mediate the cyclic AMP and [^{14}C]-glycogen responses in L_6 and C_2C_{12} cells.

Other evidence also indicates that glycogen metabolism in L_6 cells is mediated via CGRP receptors but not by amylin receptors. The ability of both CGRP (1 nM) and amylin (100 nM) to stimulate cyclic AMP accumulation in L_6 cells was not affected by incubation with 1 μM salmon calcitonin$_{8-32}$ (sCT_{8-32}), but was completely blocked by incubation with 1 μM h$\alpha CGRP_{8-37}$ (results not shown). sCT_{8-32}, compared to h$\alpha CGRP_{8-37}$, is a more potent antagonist of amylin action in isolated soleus muscle and *in vivo*, consistent with its higher affinity to rat nucleus accumbens amylin receptors (Beaumont *et al.*, 1995b). On the other hand, sCT_{8-32} has a very low affinity for CGRP receptors and does not block CGRP-ergic actions (hypotensive responses) that can be blocked by human α-CGRP (h$\alpha CGRP_{8-37}$) which has a much higher affinity for CGRP receptors. The absence of coupled amylin receptors in

L_6 cells is supported by the observation that sCT_{8-32} (1 μM) failed to inhibit cyclic AMP generation in L_6 cells following either CGRP (1 nM) or amylin (100 nM).

Rat L_6 cells and mouse C_2C_{12} cells display several characteristics of differentiated skeletal muscle. L_6 and C_2C_{12} cells fuse into multinucleated cross-striated myotubes which express creatine kinase, myokinase and glycogen-metabolizing enzymes (Yaffe, 1968; Shainberg *et al.*, 1971). The similar dose-response relationship in L_6 and C_2C_{12} cells between elevation of cyclic AMP and inhibition of [^{14}C]-glycogen formation (Figure 2) was also seen for these responses, as well as for activation of glycogen phosphorylase, in intact soleus muscle (Pittner *et al.*, 1995a). This parallelism fits with the notion that amylin and CGRP affect glycogen metabolism in muscle cell lines and in rat soleus muscle by similar mechanisms – stimulation of adenylyl cyclase-coupled receptors with resulting activation of glycogen phosphorylase and inhibition of glycogen synthase.

In conclusion, the receptors that mediate effects of amylin in intact rat soleus muscle and in rodent-derived muscle cell lines are pharmacologically distinct. The effects of amylin, CGRP, and calcitonin on soleus muscle glucose metabolism appear to be mediated by activation of adenylyl-cyclase coupled receptors, which are similar in pharmacological profile to high affinity amylin receptors in rat brain. In contrast, the effects of these peptides on glucose metabolism in the rodent muscle cell lines C_2C_{12} and L_6 appear to be mediated by activation of adenylyl cyclase-coupled CGRP receptors; thus these cell lines may be useful for studying CGRP receptors, but are not a model system for amylin action.

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