SPECIAL REPORT

Regulation of muscle glycogen metabolism by CGRP and amylin: CGRP receptors not involved

¹Kevin Beaumont, Richard A. Pittner, Candace X. Moore, Deborah Wolfe-Lopez, Kathryn S. Prickett, Andrew A. Young & Timothy J. Rink

Amylin Pharmaceuticals, Inc., 9373 Towne Centre Drive, San Diego, CA 92121, U.S.A.

The aim of the present study was to determine whether amylin and calcitonin gene-related peptide (CGRP) act through shared or distinct receptors to inhibit insulin-stimulated incorporation of [¹⁴C]-glucose into glycogen. Rat amylin was 3 fold more potent than either rat α CGRP or rat β CGRP at reducing glycogen synthesis from [¹⁴C]-glucose in insulin-treated rat soleus muscle. This action was blocked by peptide antagonists, with the rank order of potency being AC187 > salmon calcitonin_{8–32} (sCT_{8–32}) > h- α CGRP_{8–37} for antagonism of either amylin or CGRP. The antagonist potency order correlated with affinity for amylin receptors measured in rat nucleus accumbens but not CGRP receptors measured in rat L6 muscle cells. Inhibition of glucose incorporation into glycogen by amylin and CGRP appears to be mediated by shared receptors that have the pharmacological characteristics of amylin receptors, and are distinct from previously described CGRP receptors.

Keywords: Amylin; CGRP; diabetes; insulin resistance; glycogen; glucose metabolism; peptide receptors; calcitonin

Introduction Amylin is a 37 amino acid peptide that is released from pancreatic β -cells in response to feeding, and has been proposed as an endocrine partner to insulin in the regulation of carbohydrate disposal and storage (Pittner *et al.*, 1994). It shares 41–51% sequence identity with calcitonin gene-related peptide (CGRP), a neuropeptide that is widely distributed in peripheral motor and sensory nerves. Both human amylin and rat α CGRP potently inhibit insulin-stimulated glycogen synthesis in rat skeletal muscle (Leighton & Cooper, 1988). However, amylin is 100 fold less potent than CGRP in competing for [¹²⁵I]-CGRP binding sites in membranes from rat skeletal muscle (Chantry *et al.*, 1991) or rat L6 skeletal muscle cells (Poyner *et al.*, 1992).

We have identified amylin receptors in rat brain, concentrated in the nucleus accumbens, which have a binding profile that is quite distinct from CGRP receptors (Beaumont *et al.*, 1993). These amylin receptors have high affinity for AC187 (Acetyl-[Asn³⁰, Tyr³²]-sCT_{8–32}), a potent and selective antagonist of amylin-induced hyperglycaemia and hyperlactaemia (Young *et al.*, 1994), and have moderate affinity for h- α CGRP_{8–37}, which antagonizes actions of both amylin and CGRP (Wang *et al.*, 1991).

In the studies described here, we addressed the question of whether amylin and CGRP act through common or distinct receptors to inhibit muscle glycogen synthesis.

Methods *Receptor binding* Binding of [¹²⁵I]-BH-rat amylin (Bolton-Hunter labelled at the N-terminal lysine) to membranes from the nucleus accumbens of male Harlan Sprague Dawley rats (180–250 g) was measured as previously described (Beaumont *et al.*, 1993), in a modified binding buffer consisting of 20 mM NaHEPES, pH 7.4, 1 mM MgCl₂, 1 mg ml⁻¹ bacitracin, 1 mg ml⁻¹ BSA, 1 μ g ml⁻¹ phosphoramidon, and 5 μ g ml⁻¹ bestatin. To measure CGRP receptor binding, rat L6 cell membranes were incubated with 28 pM [¹²⁵I]-h- α CGRP (¹²⁵I-labelled at his₁₀) and unlabelled peptides, with buffer and experimental methods as described for amylin receptor binding.

Radioiodinated peptides were from Amersham (Arlington Heights, IL, U.S.A.). Amylin, CGRP, and h- α CGRP_{8–37} were from Bachem (Torrance, CA, U.S.A.). AC187 and sCT_{8–32} were synthesized as described (Young *et al.*, 1994).

[¹⁴C]-glucose incorporation into muscle glycogen Incorporation of [¹⁴C]-glucose into glycogen in soleus muscle strips from fasted rats was measured essentially as previously described (Young *et al.*, 1992). A 4-parameter logistic function was fitted to the data by a curve-fitting programme (InPlot, GraphPad Software, San Diego, CA, U.S.A.) to derive IC₅₀s and 95% confidence intervals (CIs). Incubations were performed in quadruplicate with muscles randomly assigned from separate animals. Results are means $\pm$ s.e.mean of data from 4–36 muscle strips measured in the indicated number of experiments. Data were analyzed for statistical significance by analysis of variance (ANOVA) and post-hoc Bonferroni test.

Results *Amylin receptor binding* To determine amylin receptor affinity, we measured the ability of peptides to inhibit binding of [¹²⁵I]-BH-rat amylin to rat nucleus accumbens membranes. As reported previously (Beaumont *et al.*, 1993), rat amylin was more potent than rat α CGRP or rat β CGRP in competing for [¹²⁵I]-BH-rat amylin binding (Table 1). Among the antagonists, AC187 was the most potent inhibitor of [¹²⁵I]-BH-amylin binding, with an IC₅₀ = 0.48 nM (Table 1).

CGRP receptor binding To determine CGRP receptor affinity, we measured the ability of peptides to compete for [¹²⁵I]-h- α CGRP binding to rat L6 cell membranes (Poyner *et al.*, 1992). Rat amylin was considerably less potent than rat α CGRP or rat β CGRP in competing for [¹²⁵I]-h- α CGRP binding (Table 1). Of the antagonists, h- α CGRP_{8–37} was by far the most potent inhibitor of CGRP receptor binding with an IC₅₀ = 1.1 nM. For all peptides, IC₅₀s for inhibition of [¹²⁵I]-h- α CGRP binding differed significantly from IC₅₀s for inhibition of [¹²⁵I]-BH-rat amylin binding ($P < 0.05$, Student's unpaired *t* test).

Functional responses to amylin and CGRP in soleus muscle Rat amylin (EC₅₀ = 5 nM, 95% CI = 2.2–11 nM, $n = 9$) was 3 fold more potent than rat α CGRP (EC₅₀ = 15 nM, 95%

¹ Author for correspondence.

Table 1 Inhibition by peptides and antagonists of [125 I]-BH-rat amylin binding to rat nucleus accumbens membranes and of [125 I]-hCGRP- α binding to L6 cell membranes

Compound	[125 I]-BH-rat amylin rat n. accumbens (IC ₅₀ nM)	[125 I]-hCGRP- α rat L6 cells (IC ₅₀ nM)
Agonists		
Rat amylin	0.054 $\pm$ 0.021 (3)	4.2 $\pm$ 0.5 (3)
Rat α CGRP	0.656 $\pm$ 0.130 (3)	0.092 $\pm$ 0.021 (3)
Rat β CGRP	0.105 $\pm$ 0.015 (3)	0.030 $\pm$ 0.011 (3)
Antagonists		
AC187	0.48 $\pm$ 0.16 (5)	73 $\pm$ 20 (3)
sCT ₈₋₃₂	1.9 $\pm$ 0.37 (7)	2700 $\pm$ 1400 (3)
h- α CGRP ₈₋₃₇	13 $\pm$ 6.2 (4)	1.1 $\pm$ 0.22 (3)

Results are means $\pm$ s.e.mean for the indicated number of separate experiments.

CI = 7–32 nM, n = 3) or rat β CGRP (EC₅₀ = 14 nM, 95% CI = 12–16 nM, n = 3) at reducing the incorporation of [14 C]-glucose into glycogen in insulin-treated soleus muscles from fasted rats (Figure 1).

Insulin-treated soleus muscles were incubated with 100 nM rat amylin or 100 nM rat α CGRP in the presence of increasing concentrations of antagonists, resulting in reversal of the inhibition of [14 C]-glucose incorporation into glycogen (Figure 1). IC₅₀s (with 95% confidence intervals) for antagonizing rat amylin were AC187 = 0.10 μ M (0.043–0.23 μ M, n = 4), sCT₈₋₃₂ = 0.78 μ M (0.22–2.8 μ M, n = 2), and h- α CGRP₈₋₃₇ = 5.7 μ M (3.2–9.9 μ M, n = 6). IC₅₀s for antagonizing rat α CGRP were AC187 = 0.26 μ M (0.030–2.3 μ M, n = 2), sCT₈₋₃₂ = 1.6 μ M (0.23–12 μ M, n = 2) and h- α CGRP₈₋₃₇ = 30 μ M (12–78 μ M, n = 2). The antagonists had similar rank order potencies (AC187 > sCT₈₋₃₂ > h- α CGRP₈₋₃₇) in blocking either rat amylin or rat α CGRP. This rank order of potency corresponded to the rank order for inhibiting amylin binding sites in rat nucleus accumbens, and was quite distinct from the rank order for inhibiting CGRP receptor binding in rat L6 cells.

Discussion These results indicate that amylin and CGRP act through a common receptor population to alter glycogen metabolism in rat skeletal muscle. The evidence for this conclusion is pharmacological, based on the similar potencies of antagonists at inhibiting the actions of either rat amylin or rat α CGRP. The relative potency of antagonists strongly suggests that CGRP receptors do not mediate the effects of either CGRP or amylin on muscle glucose metabolism. Instead, the effects of both amylin and CGRP are mediated by receptors that have high affinity for AC187 and sCT₈₋₃₂, like the amylin receptors concentrated in the nucleus accumbens region.

Data available from functional studies are consistent with the idea that the effects of amylin on glycogen phosphorylase and glycogen synthase are produced through stimulation of an adenyl cyclase-coupled receptor. Amylin stimulates increased cyclic AMP in intact rat soleus muscle with an EC₅₀ = 0.3 nM (Pittner *et al.*, 1994).

The pharmacological evidence described here indicates that CGRP receptors measured by [125 I]-CGRP binding to rat muscle membranes (Chantry *et al.*, 1991) do not regulate muscle glucose metabolism. One function that has been proposed for CGRP receptors in skeletal muscle is regulation of nicotinic cholinergic expression and sensitivity (New &

Figure 1 (a) Inhibition of insulin-stimulated [14 C]-glucose incorporation into glycogen in isolated soleus muscles by rat amylin (O), rat α CGRP (square), or rat β CGRP (triangle). Rat amylin differed significantly (P < 0.05, ANOVA) from rat α CGRP at 3, 10, and 100 nM and from rat β CGRP at 10 and 100 nM. Inhibition of the effects of (b) 100 nM rat amylin or (c) 100 nM rat α CGRP on [14 C]-glucose incorporation into soleus muscle glycogen by AC187 (O), sCT₈₋₃₂ (square), or h- α CGRP₈₋₃₇ (triangle). AC187 differed significantly (P < 0.05, ANOVA) from h- α CGRP₈₋₃₇ at 0.1 and 1 μ M. Means $\pm$ s.e.mean of at least 4 soleus muscle strips.

Mudge, 1986). CGRP receptors may also be present in vascular elements in membranes prepared from skeletal muscles.

In conclusion, inhibition of muscle glycogen synthesis by

amylin and CGRP appears to be mediated by shared receptors that have pharmacological characteristics of amylin receptors, not CGRP receptors.

References

- BEAUMONT, K., KENNEY, M.A., YOUNG, A.A. & RINK, T.J. (1993). High affinity amylin binding sites in rat brain. *Mol. Pharmacol.*, **44**, 493–497.
- CHANTRY, A., LEIGHTON, B. & DAY, A.J. (1991). Cross-reactivity of amylin with calcitonin-gene-related peptide binding sites in rat liver and skeletal muscle membranes. *Biochem. J.*, **277**, 139–43.
- LEIGHTON, B. & COOPER, G.J. (1988). Pancreatic amylin and calcitonin gene-related peptide cause resistance to insulin in skeletal muscle in vitro. *Nature*, **335**, 632–635.
- NEW, H.V. & MUDGE, A.W. (1986). Calcitonin gene-related peptide regulates muscle acetylcholine receptor synthesis. *Nature*, **323**, 809–811.
- PITTNER, R.A., ALBRANDT, K., BEAUMONT, K., GAETA, L.S.L., KODA, J.E., MOORE, C.X., RITTENHOUSE, J. & RINK, T.J. (1994). Molecular physiology of amylin. *J. Cell. Biochem.*, **55S**, 19–28.
- POYNER, D.R., ANDREW, D.P., BROWN, D., BOSE, C. & HANLEY, M.R. (1992). Pharmacological characterization of a receptor for calcitonin gene-related peptide on rat, L6 myocytes. *Br. J. Pharmacol.*, **105**, 441–447.
- WANG, M.W., YOUNG, A.A., RINK, T.J. & COOPER, G.J. (1991). $^{8-37}$ h-CGRP antagonizes actions of amylin on carbohydrate metabolism in vitro and in vivo. *FEBS Lett.*, **291**, 195–8.
- YOUNG, A.A., GEDULIN, B., GAETA, L.S.L., PRICKETT, K.S., BEAUMONT, K., LARSON, E. & RINK, T.J. (1994). Selective amylin antagonist suppresses rise in plasma lactate after intravenous glucose in the rat. *FEBS Lett.*, **343**, 237–241.
- YOUNG, A.A., GEDULIN, B., WOLFE-LOPEZ, D., GREENE, H., RINK, T.J. & COOPER, G.S. (1992). Amylin and insulin in rat soleus muscle: dose-responses for co-secreted non-competitive antagonists. *Am. J. Physiol.*, **263**, E274–E281.

(Received January 27, 1995)

Accepted March 30, 1995)