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A cysteine-scan of the N-terminus of calcitonin gene-related peptide

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The N-terminus of calcitonin gene-related peptide (CGRP) is essential for receptor activation, but its structure-activity relationship is poorly defined. A cysteine scan of residues 1 to 9 of human (h) αCGRP has been attempted, excluding the native cysteines at positions 2 and 7, primarily to indicate where cysteines can be introduced for derivatisation, but also to probe structure-activity relationships.

Peptides were synthesised by Alta Bioscience (Birmingham, UK). Purification was by hplc and target identification was by MALDI-TOF and electrospray mass spectroscopy. The activity of the peptides was assayed on SK-N-MC cells which express an endogenous CGRP receptor, measuring the pEC_{50} for cAMP production as described previously (Poyner *et al.,* 1998), using Graphpad Prism 4.00 for data-fitting. To assess if Emax values differed from wild-type (100), 95% confidence limits were calculated using Student's t statistic.

The Cys-1 derivative showed a limited (10-fold) reduction potency with a slight reduction in the maximum response. There were problems in producing some peptides, especially Cys3- and Cys5-hα CGRP (Table 1). In spite of its low purity, the Cys3-containing mixture gave an activity very similar to that of native CGRP, although the interpretation of this is problematic. The Cys8- and Cys9- derivatives appeared to be partial agonists, although their low purity means caution is needed in interpreting this data. At concentrations up to 1 αM, the Cys6- derivative failed to stimulate cAMP production. Instead, it caused a rightward shift in the dose response curve to CGRP with no change in the maximum response (106 ± 5% of that seen with CGRP) but the pEC_{50} was shifted to 7.54 ± 0.10 (n=3), consistent with it being an antagonist with a pA_2 of 7.2.

Table 1. Ability of CGRP derivatives to stimulate cAMP production

Peptide	**Purity**	**pEC_{50}**	**Emax (%)**	**Peptide**	**Purity**	**pEC_{50}**	**Emax (%)**
hα-CGRP	>95%	8.80±0.09	100	[Cys6]-hαCGRP	>95%	<6	ND
[Cys1]-hαCGRP	>95%	7.70±0.28	71±8*	[Cys8]-hαCGRP	>80%	7.00±0.74	30±10*
[Cys3]-hαCGRP	<50%	8.94±0.13	94±4	[Cys9]-hαCGRP	>70%	6.66±0.38	55±11*
[Cys5]-hαCGRP	<50%	<6	ND				

*Values are means ± s.e.m, n=3. ND, not detectable. *, 95% confidence limit excludes 100*

This work suggests that cys-substitution at the extreme N-terminus of CGRP is best tolerated. Replacement of Thr6 by cysteine produces an antagonist; this threonine is conserved in all members of the CGRP family of peptides (calcitonin, amylin adrenomedullin, adrenomedullin 2) and so may be important for receptor activation. An alanine scan of the N-terminus of CGRP would be useful to confirm these conclusions.

Poyner, D.R. *et al.,* (1998), *Br. J. Pharmacol,* 124, 1659-1666.

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