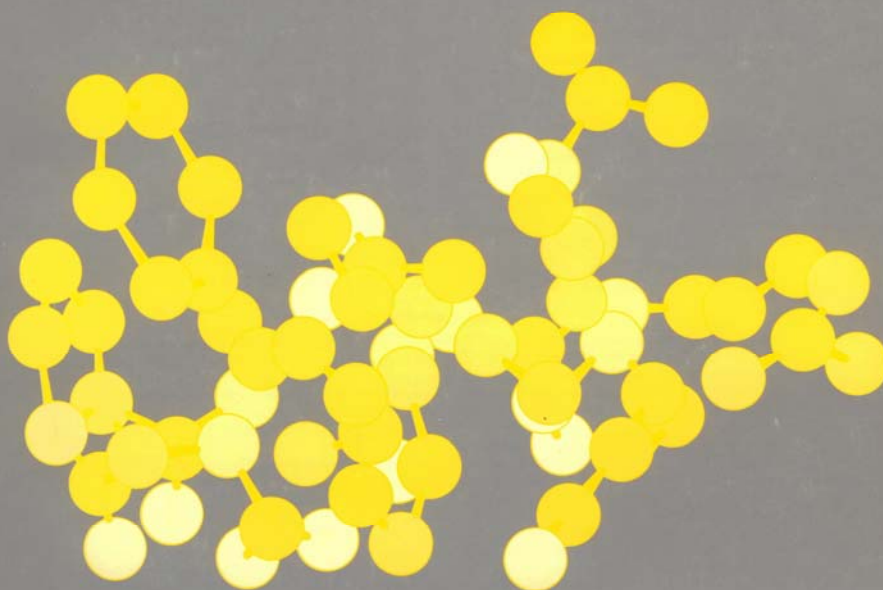


# Regulatory Peptides

---



ELSEVIER

11 JUNE 1992 VOLUME 39 NUMBERS 2-3

last issue of this volume  
ISSN 0167-0115  
REPPDY 39 123-290 (1992)

## SYNTHESIS AND BIOLOGICAL APPLICATION OF A FLUORESCENTLY LABELLED NEUROMEDIN C ANALOGUE

J. GRAY<sup>1</sup>, B. WALKER<sup>2</sup>, T. ADRIAN<sup>3</sup>, R.F. MURPHY<sup>3</sup> and J. NELSON<sup>2</sup>, <sup>1</sup>Biosyn Ltd., Medical Biology Centre, Belfast BT9 7BL. <sup>2</sup>Division of Biochemistry, The Queen's University of Belfast, UK. <sup>3</sup>School of Medicine, Creighton University, Omaha, Nebraska, USA

Following a recent publication reporting the use of a biotinylated GRP derivative as a receptor probe, we report here on the synthesis, characterisation and biological application of 4(5)-carboxyfluorescein-neuromedin C. This fluorescently labelled receptor probe would be better suited to the desired needs of a cell biology tool, as it requires a less complicated staining procedure and would have better potential applications in flow cytometry and investigations of receptor internalisation.

This peptide was synthesised using the Fmoc/solid phase methodology and the reporter group was coded *in situ*. The labelled peptide was purified by HPLC and its structure confirmed by amino acid analysis.

When tested in an *in vitro* amylase release assay on dispersed guinea pig pancreatic acinar cells, 4(5)-carboxyfluorescein-neuromedin C stimulation followed a dose-dependent pattern similar to that of the parent peptide with a half-maximal stimulation at 200nM (20nM for neuromedin C). Use of the probe was demonstrated by UV microscopy on Cos-7 monkey kidney cells *in vitro* (known to express high levels of bombesin/GRP receptors) and on rat stomach cryostat sections, where the binding pattern was confirmed by microautoradiography. Fluorescent labelling was maximal at 10 $\mu$ M probe application but fainter labelling could still be observed at concentrations as low as 0.1 $\mu$ M. A 1000-fold molar excess of neuromedin C blocked the fluorescent probe labelling.

## VIP AND PACAP STIMULATION OF DUODENAL MUCOSAL AND BRUNNER'S GLAND ADENYLATE CYCLASE ACTIVITY

S. HAMILTON<sup>1</sup>, B.H. HIRST<sup>1</sup>, N.L. SIMMONS<sup>1</sup> and M.E. PARSONS<sup>2</sup>.

<sup>1</sup>Department of Physiological Sciences, University of Newcastle upon Tyne, Medical School, Newcastle upon Tyne NE2 4HH, U.K. and <sup>2</sup>SmithKline Beecham, The Frythe, Welwyn, U.K.

Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase activating peptide (PACAP) have been localized to the duodenum. We have compared the adenylate cyclase stimulating activity of these and related peptides in rat homogenized submucosal Brunner's glands of the duodenum and the overlying mucosal tissue of the duodenal bulb. Separation of Brunner's and mucosal tissue was confirmed by alkaline phosphatase activity in the two tissues; specific activity was 12-fold greater in the mucosa than Brunner's tissue, consistent with histochemical visualization. Basal adenylate cyclase activity was consistently greater in Brunner's as compared with mucosal tissue (257 $\pm$ 24 (n=17) and 96 $\pm$ 16 (n=15) pmol cAMP/mg protein/15min, respectively). Forskolin 10<sup>-4</sup>M increased adenylate cyclase activity above basal in both tissues, >2-fold greater in Brunner's than mucosal tissue (1333 $\pm$ 144 (n=16) and 562 $\pm$ 52 (n=13) pmol cAMP/mg/15min, respectively). In duodenal mucosal tissue, PACAP-38 10<sup>-6</sup>M increased adenylate cyclase activity (480 $\pm$ 69 (n=4) pmol cAMP/mg/15min) approaching the response to forskolin, with an EC<sub>50</sub> ~ 5x10<sup>-9</sup>M. The rank order for stimulation of adenylate cyclase was (PACAP-38=VIP=PHI=PACAP-27) > GRF > secretin, with no significant stimulation with glucagon 10<sup>-6</sup>M. PACAP(6-27), 3 $\mu$ M, antagonised the response to PACAP-38 to a greater extent than [4-Cl-D-Phe<sup>6</sup>, Leu<sup>17</sup>]VIP. In Brunner's tissue, PACAP-38 10<sup>-6</sup>M (242 $\pm$ 65 (n=4) pmol cAMP/mg/15min) and other peptides resulted in increases in adenylate cyclase activity of only 20% of that with forskolin, at concentrations  $\geq$  10<sup>-8</sup>M.

## NEUROPEPTID

M.R. ELPHICK<sup>\*</sup>  
London, Egham  
Sciences, Univer

HPLC of wh  
immunoreactive  
Elphick *et al.*, 19  
nonapeptide, FPV  
C and D1 are un  
Immunocytoch  
distribution of SA  
anterior segment c  
with the basi-epit  
of connective tiss  
SALMFamide-li  
epithelial nerve pl  
In contrast to our  
neuroendocrine ce  
It seems likely I  
associated with I  
(FPVGRVHRFamide  
presently raising sq  
map of its distribut  
In addition, we  
expression during s

## ACTIVITY OF VASO RECEPTORS ON ISC

D Bell and B J McDerm  
Belfast, Belfast BT9 7BE

In contrast to the hearts  
proposed that the rat he  
receptor\* (1). This early  
membranes. Such suspe  
ventricular cell types. TI  
secretin and VIP on the i  
demonstrate a direct stim  
mediated by a selective ri

In the presence of adenos  
(1 mM), both secretin an  
concentration-dependant  
concentrations, secretin (1  
1.3 fold elevation was obs

The selective antagonist fe  
the action of VIP (50 nM)  
(1-100  $\mu$ M), the concentrat  
for secretin 7-27 was 4.89.  
response curve. VIP (10  $\mu$ i  
cAMP production at high  
cardiomyocytes are devoid  
VIP behaves as a partial ag  
Chatelain, P *et al* (1980). P