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Short communication

CALCITONIN GENE-RELATED PEPTIDE IS A POTENT INHIBITOR OF SUBSTANCE P DEGRADATION

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Calcitonin gene-related peptide (CGRP) was found to potently inhibit a substance P endopeptidase isolated from human CSF CGRP potentiated substance P irritant actions, a possible mechanism is interaction for a common metabolic step Somatostatin is another peptide capable of competing with substance P endopeptidase

Calcitonin gene-related peptide Substance P endopeptidase Somatostatin Substance P

1. Introduction

A new peptide, calcitonin gene-related peptide (CGRP) is formed in nervous tissue by alternative splicing of the primary RNA-transcript of the calcitonin gene (Amara et al., 1982). CGRP has been localized by immunohistochemistry in several areas of the central and peripheral nervous system, including small diameter sensory fibers (Rosenfeld et al. 1983) CGRP and substance P (SP) seem to be co-localized in a certain proportion of these sensory fibers (Gibson et al, 1984; Wiesenfeld-Hallin et al, 1984). SP has gradually reached the status of a putative neurotransmitter in sensory nerves (see Pernow, 1983). Intrathecal substance P also seems to act as an irritant in rats, causing caudally directed biting and scratching behaviour when applied intrathecally at the lumbar level (Hyldén et al., 1981; Piercey et al., 1981, Seybold et al., 1982)

Wiesenfeld-Hallin et al (1984) recently observed that aversive behaviours induced by SP were potentiated by CGRP at doses which were not themselves active. The mechanism of this effect remained unknown. One possibility could be interaction with a common degradation mechanism There are many enzymes known to degrade SP Since relatively high concentrations of the SP-(1-7) fragment have been found in rat spinal cord (Sakurada et al., 1985) this fragmentation may be an important route of degradation. We therefore tested the interaction of CGRP with SP degradation by an enzyme isolated from human CSF, which generates the SP-(1-7) fragment (Nyberg et al., 1984)

2. Materials and methods

Substance P, rat(r) and human(h) CGRP and Tyr-rCGRP-(23-37) were from Bachem, Bubendorf, Switzerland. Somatostatin and vasopressin were from Kabi AB, Stockholm, Sweden, and Sigma, St Louis, MO, U S A., respectively. SP-(1-7) was prepared by Dr. J M. Stewart, Dept of Chemistry, University of Colorado, Denver, CO The substance P endopeptidase was isolated from human CSF as previously described Purification of the enzyme was monitored by the formation of SP-(1-7) which was measured by radioimmunoas-

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say (Nyberg et al 1984) The specific activity of the enzyme used in the present experiments was about 3.7 nmol SP-(1-7) min^{-1} mg $protein^{-1}$

Degradation experiments were conducted in a total volume of 50 µl of a 0.02 M Tris-HCl buffer, pH 7 8 at 37°C. The incubation was terminated by boiling for 2 min and the sample was diluted with 0 5 ml methanol before centrifugation and evaporation The product, SP-(1-7), was measured by a specific radioimmunoassay (cross-reaction, with SP, C-terminal fragments of SP, SP-(1-4) and SP-(1-6) < 0.2%, with SP-(1-8) 1%) as described elsewhere (Nyberg et al., 1984). CGRP and other neuropeptides were tested for their capacity to inhibit the conversion of SP to the SP-(1-7) fragment They did not interfere in the radioimmunoassay

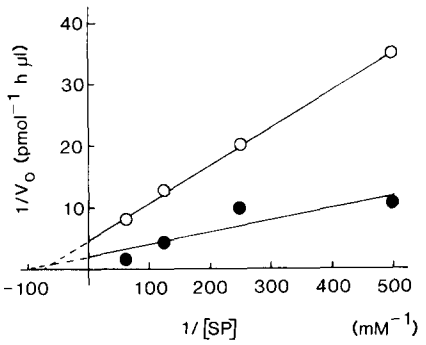

Fig 1 Lineweaver-Burk plot of the kinetics of SP endopeptidase activity in the absence (filled circles) or presence (open circles) of 20 µM rCGRP

3. Results

rCGRP caused a dose-dependent inhibition of SP-(1-7) formation Somatostatin also was active, whereas vasopressin was completely inactive at comparable concentrations (table 1). In separate experiments, hCGRP was found to dose dependently suppress the enzymatic conversion whereas the synthetic peptide Tyr-rCGRP-(23-37) was inactive (not shown)

A kinetic study of the interaction between SP and CGRP is shown in fig 1 The data indicate a non-competitive interaction. The K_m for SP calculated by linear regression is 11×10^{-5} M and the K_i for rCGRP (assuming non-competitive interaction) is $1\,6 \times 10^{-5}$ M Due to limited access to enzyme (and CSF) a more complete analysis was not possible

TABEL 1
Effect of several neuropeptides on the formation of SP-(1-7) from SP by an endopeptidase isolated from human CSF The substrate, SP, was present at a concentration of 0 8 µM Incubation was for 30 min Data are means from two separate experiments

Concentration (µM)	Inhibition (%)		
	rCGRP	Somatostatin	Vasopressin
40	95	83	0
4	63	6	0
0 4	9	0	0

4. Discussion

The starting point for this investigation was the observation by Wiesenfeld-Hallin et al (1984) that rCGRP potentiated the irritant effects of intrathecally administered SP in the rat One possible explanation would be interaction with a common elimination step Action of the endopeptidase described here or an enzyme with similar specificity isolated from brain (Lee et al, 1981), would eliminate typical SP activity in most systems The SP-(1-7) fragment can be found in the rat CNS, where it occurs in particularly high concentrations in the spinal cord (Sakurada et al, 1985) Our data therefore support the hypothesis. The SP endopeptidase used here has negligible activity against the related peptides, neurokinin A and B, and several other peptides including [Leu^5]enkephalin and β-casomorphin (Nyberg et al., 1984) The interaction with CGRP and somatostatin, both cyclic peptides, was quite unexpected. The third cyclic peptide tested, vasopressin, was inactive which also emphasized the specificity of the effect. Characterization of CGRP and somatostatin degradation products is needed to identify the sequence specificity of the enzyme.

It has recently been recognized that neurons

often contain more than one class of synaptic messengers, and different types of interaction between presumably co-released compounds have been described (see Lundberg and Hokfelt, 1983) The present findings indicate a new type of interaction of two peptides possibly released together at the central branches of primary sensory neurons in the dorsal horn of the spinal cord Thus, one peptide (CGRP) may potentiate the action of the other peptide (SP) by inhibiting its breakdown. In a physiological perspective, it is also striking that one enzyme seems to recognize three structurally very different peptides, all of which are known to be present in small diameter primary afferent nerves

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