CORTICOTROPHIN-RELEASING FACTOR - MEDIATED ADRENOCORTICOTROPHIN RELEASE FROM RAT ANTERIOR PITUITARY CELLS IS POTENTIATED BY C-TERMINAL GASTRIN-RELEASING PEPTIDE


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ABSTRACT

The remission of Cushing's syndrome following surgical removal of a tumour containing bombesin-like immunoreactivity (BLI), but insignificant levels of ACTH, is described. However, an acid extract of the tumour tissue caused the release of ACTH from isolated rat anterior pituitary cells in vitro. These observations led to an investigation of the effects of synthetic C-terminal gastrin-releasing peptide (GRP(14-27)) on ACTH release from isolated rat anterior pituitary cells. GRP(14-27) (10-1000 ng/ml) directly stimulated the release of ACTH in vitro, whereas lower doses (10-1000 pg/ml), ineffective themselves in eliciting ACTH release, potentiated the CRF-mediated in-vitro release of ACTH.

INTRODUCTION

The amphibian skin tetradecapeptide bombesin (Anastasi, Erspamer & Bucci, 1971) and its mammalian counterpart, the porcine heptacosapeptide gastrin-releasing peptide (GRP) (Fig. 1) (McDonald, Jornvall, Nilsson et al. 1979) have been shown to elicit a wide variety of responses from the mammalian gastro-intestinal tract and central nervous system (Erspamer, Melchiorri, Falconier-Erspamer & Negri, 1978; McDonald, 1981). The presence of bombesin-like immunoreactivity (BLI) in the mammalian hypothalamus (Brown, Allen, Villarreal et al. 1978) and the identification of pituitary cell bombesin receptors (Westendorf & Schonbrunn, 1983) indicate a possible role for bombesin-like peptides in the control of the pituitary. The effects of bombesin on anterior pituitary hormone secretion are controversial. In the rat it has been reported to stimulate growth hormone and prolactin release both in vivo (Rivier, Rivier & Vale, 1978) and in vitro (Westendorf & Schonbrunn, 1982). However, in man, although prolactin release has been reported (Panttirolli, Alberetto, Restelli & Fachiineti, 1980), there has been a failure to demonstrate any effect of bombesin on the secretion of a number of other anterior pituitary hormones (Morley, Varner, Modlin et al. 1980).

AMPHIBIAN BOMBESIN

1pGlu-Gln-Arg-Leu-Val-Gly-His-Leu-Met-NH2

PORCINE GRP

14Met-Tyr-Pro-Ar g-G1y-A1n-His-Trp-Ala-Val-G1y-His-Leu-Met-NH2

Lys

14

Ala-Leu-Val-Thr-Gly-G1y-Gly-Val-Ser-Val-Pro-Ala

Fig. 1. Amino acid sequences of amphibian bombesin and porcine GRP, indicating 'C' terminal homology.

A 41-year old man presented with classical symptoms of the ectopic adrenocorticotrophin (ACTH) syndrome associated with metastatic carcinoma of the thyroid. Pre-operative plasma ACTH ranged from 35-185 ng/l (normal range (NR) <80 ng/l), calcitonin (CT) was 49 μg/l (NR <20 μg/l) and BLI was 75 ng/l (NR <5 ng/l). Plasma arginine vasopressin (AVP) was normal. After partial removal of metastatic tumour tissue from the mediastinum, plasma ACTH and BLI levels returned to normal and his Cushing's syndrome resolved.

MATERIALS AND METHODS

Tumour tissue removed at surgery was collected into sterile Earle's Balanced Salt Solution (EBSS) for in-vitro studies, or frozen on dry ice. Frozen tissue was thawed, extracted in 10 mM-HCl (100 mg wet weight/ml) and assayed for immunoreactive ACTH, β-lipotrophin (B-LPH), β-endorphin (β-EP), γ-melanotrophin (γ-MSH), corticotrophin-releasing factor (CRF), somatostatin (SS), AVP and BLI. Tissue was immunostained for ACTH, B-LPH, β-EP, γ-MSH, CRF, BLI and CT using an indirect immunoperoxidase method.

The secretion of ACTH from the tumour was investigated in the isolated perfused tumour cell column (Gillies, Ratter, Grossman et al. 1980). Cells were dispersed by trypsinization, 1×10⁶ cells per column mixed with a Biogel P2 matrix and perfused at 0.5 ml/min with EBSS containing 0.25% human serum albumin, Trasylol and antibiotics. After an equilibration period of 90 min, the isolated tumour cells were stimulated with synthetic ovine CRF-41 (Dr D. Coy) (10-90 ng/mg) in 3-min pulses. Two-min fractions were collected and assayed for ACTH.

The ACTH releasing bioactivity of the tumour tissue and synthetic GRP(14-27) was studied using the isolated rat anterior pituitary cell column (Gillies & Lowry, 1978). Cells were prepared as described for tumour cells. Pulsed doses of the acid extract of the tumour were compared with synthetic oCRF-41 (10-90 ng/ml) and the CRF activity of a rat stalk median eminence extract (SME). GRP(14-27) was tested alone in doses from 10-1000 ng/ml.

Two columns were set up in parallel, one as control to examine the effect of a continuous perfusion of GRP(14-27) at doses which, in themselves (10-1000 pg/ml), did not cause ACTH release. After stimulation of each column with doses of oCRF-41 (10-90 pg/ml), GRP(14-27) was added to the perfusion medium of the test column. Both columns were stimulated with 3-min pulses of oCRF-41 (30 ng/ml) which continued after the infusion of GRP has ceased.

Finally columns were stimulated with 3-min pulses of oCRF-41 (10-90 ng/ml) alone, or with a constant dose of GRP(14-27) at a sub-threshold level.

RESULTS

Peptide levels in the tumour extract. The concentration of ACTH (0.018 ng/mg) was considerably less
than normally found in ectopic ACTH-secreting tumours (1.03±0.63 ng/mg) (Ratcliffe, Knight, Besser et al. (1972). CRF, AVP and SS levels were all undetectable. BLI was 0.56 ng/mg.

Immunostaining Immunostaining was negative for ACTH and related peptides, CRF and SS, but positive for calcitonin and BLI.

Isolated tumour cell column ACTH was undetectable in all fractions from the tumour cell column.

Bioactivity of tumour extract on isolated rat pituitary cells Stimulation of the cells with tumour extract produced an increase in ACTH release of 500% above basal levels, correcting for the ACTH content of the tumour tissue. This response was very weak in comparison with the CRF bioactivity of rat stalk median eminence extract (SME), and with the bioactivity of synthetic oCRF-41 (Fig. 2).

Bioactivity of GRP(14-27) on isolated rat pituitary cells Supraphysiological doses of synthetic GRP(14-27) (10-1000 ng/ml) stimulated ACTH release in a linear dose-dependent manner (Fig. 3). The BLI content of tumour extract producing a 500% increase over basal level was 56 ng.

Fig. 2. Percentage increase in ACTH release over basal secretion in response to (●) rat SME, (▲) oCRF-41 and (●) an extract of the tumour tissue. Vertical bars represent ±1SEM of three determinations.

Fig. 3. Percentage increase in ACTH release over basal secretion in response to synthetic GRP(14-27). Vertical bars represent ±1SEM of 4 determinations in 2 separate experiments.

Continuous perfusion with GRP(14-27), in doses themselves insufficient to release ACTH (10-1000 pg/ml), in conjunction with simultaneous stimulation with 3-min pulses of CRF (30 ng/ml), resulted in a 100% increase in the ACTH response to CRF. A carry-over effect was observed, until the response returned to that of the control column. However, this effect was not dependent upon the dose of GRP(14-27) perfused (Fig. 4).

Fig. 4. ACTH release from 2 columns set up in parallel, indicating identical dose-dependent response to CRF in each column; prior to infusion of GRP(14-27). (●-●) Response of column with GRP infusion; (●-●) response of control column.
Stimulation with 3-min pulses of a combined dose of oCRF-41 (10-90 pg/ml) and GRP(14-27) (100 pg/ml) resulted in an increase of ACTH release of approximately 60% over that released by the equivalent dose of oCRF-41 alone (Fig. 5).

**Fig. 5.** Percentage increase in ACTH release over basal in response to different doses of CRF alone ( ), or in combination with GRP(14-27) (100 pg/ml) ( ). Vertical bars represent ±SEM of 3 determinations.

**DISCUSSION**

The results indicate that low doses of GRP(14-27) are capable of potentiating the ACTH response to oCRF-41. However, ACTH release could only be directly stimulated by considerably higher doses of GRP(14-27).

It is now recognized that the hypothalamic factor responsible for the control of corticotrophin release is a multifactorial complex, and not a single substance. There is, however, considerable controversy over the constituents of the CRF complex (Gillies & Lowry, 1982). The recently characterized ovine CRF-41 (Vale, Speiss, Rivier & Rivier, 1981) has been shown to stimulate the release of ACTH, ß-LPH and ß-EP from cultured anterior pituitary cells. The action of this peptide is potentiated several times by AVP (Bény & Baertschi, 1982) and also by AVP-free hypothalamic extracts, confirming that an additional factor or factors may be involved in the complex (Gillies, Linton & Lowry, 1982).

From our clinical observations and subsequent in-vitro experiments, we propose that GRP, the mammalian counterpart of amphibian bombesin, may be involved in the CRF complex and deserves further investigation.

**ACKNOWLEDGEMENTS**

We thank the Cancer Research Campaign and Medical Research Council for support and Dr P.H. Baylis for AVP measurements. We also thank Miss Sandra Burnham for preparation of the manuscript.

**REFERENCES**