vasodilatation. This confirms the axon reflex nature of the characteristic cholinergic erythema. A single application of capsaicin enhanced the urticarial response by releasing substance P, which acted synergistically with the histamine released. The absence of response to indomethacin therapy speaks against a role for prostaglandins in the pathogenesis of cholinergic urticaria.[18] Likewise, platelet studies provide no support for participation of the powerful urticariogen acetylglyceryl ether phosphonylcholine in this disease.[19] Histamine is the key effector substance.

This demonstration that cholinergic urticaria is an acetylcholine receptor disease associated with subclinical IgE sensitisation suggests the need for screening of such patients for occult antigen sensitivity. Cholinergic patch testing should prove useful. Furthermore, the concept that acetylcholine may elicit IgE-mediated hypersensitivity reactions could well be helpful in the study of such autonomically triggered diseases as atopic dermatitis and asthma.

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KATACALCIN: A NEW PLASMA CALCIUM-LOWERING HORMONE

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Summary A second potent plasma calcium-lowering peptide, katacalcin (PDN-21), flanks calcitonin within the human calcitonin precursor. Plasma katacalcin was present in 57 healthy volunteers. Concentrations were higher in males than in females and approximately equimolar with calcitonin. Plasma katacalcin doubled within 5 min of calcium infusion. Plasma katacalcin was markedly raised in 20 patients with medullary carcinoma of the thyroid. Measurement of plasma katacalcin concentrations may prove useful in the diagnosis and follow-up of this condition. Katacalcin, like calcitonin, may be involved in both plasma calcium regulation and skeletal maintenance and thus may prove useful in the treatment of bone disease.

Introduction

LIKE many other peptide hormones calcitonin (CT) is cleaved from a large precursor polyprotein. It is flanked on its C-terminal side by a 21-aminoacid peptide and on its amino-terminal side by a larger peptide, for which the complete sequence is unknown.[1,2] Professor A. P. Waterson has suggested that because of its potent calcium-lowering effect the C-terminal flanking peptide (PDN-21[3] in the chemical terminology of Tatemoto and Mutt[4]) should be called katacalcin (KC) (κατα, down). (Hypocalcin is the name given to the calcium-lowering hormone from the corpuscles of Stannius in fish.[5])

KC is one of the first examples of a new hormone discovered by use of recombinant DNA technology, rather than by the traditional techniques of tissue extraction and purification based on biological assay.

The action of KC does not depend upon the presence of the kidneys and it is also active on mouse calvariae in organ culture. However, KC does not duplicate the effect of CT; unlike CT, it has no effect on plasma phosphate. Indeed, the effects of the two hormones are additive and KC probably acts at a different receptor.[3] We have developed a radioimmunoassay for the new peptide and, together with a simple extraction procedure, this has allowed us to measure plasma concentrations in man.

Methods

Volunteers

After an overnight fast, 20–25 ml venous blood was collected from 57 normal volunteers into cooled heparinised tubes (29 males, mean age 33 years [range 22–60]; 28 females, mean age 28 years [range 18–37]). Plasma was separated and frozen within 30 min. Calcium (2 mg/kg body-weight) was infused for 1 min in 3 female and 2 male volunteers after an overnight fast and blood was collected at 0, 2, 5, 10, and 15 min. Control saline infusions were given on a different morning. All blood-samples were taken between 9 and 10.30 AM.

Patients

Plasma was obtained from 20 patients with histologically proven medullary carcinoma of the thyroid and CT and KC were measured as described below.

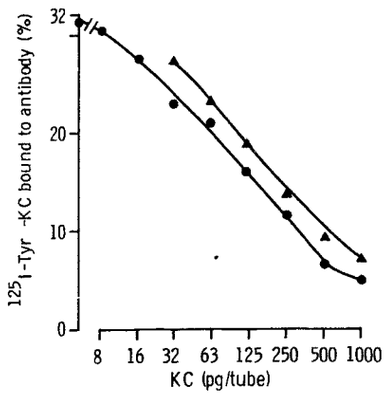

Fig 1—Typical displacement curves for (●) synthetic KC and (▲) synthetic KC taken through extraction procedure.

Calcitonin

CT was measured by either a non-equilibrium 7-day assay (sensitivity 2 pg/tube)[6] or an overnight assay (sensitivity 10 pg/tube)[7] as appropriate.

Katacalcin

Antibody.—Tyrosyl-katacalcin ([Tyr]°-KC) (Peninsula Laboratories Inc, California, USA) was conjugated to ovalbumin by means of a modification of the glutaraldehyde method[8,9] and antibodies were raised in rabbits by subcutaneous injection of the conjugate in Freund's complete adjuvant (Difco) (100 μg KC equivalent/rabbit). A booster injection was given 6 weeks later and blood was obtained after a further 7 days.

Procedure.—A radioimmunoassay was set up in which [Tyr]°-KC was iodinated and synthetic KC (Peninsula Laboratories Inc) was used as standard. At least two sample dilutions in duplicate were used for each assay. We chose either an overnight equilibrium assay (sensitivity 40 pg/tube) or a 5-day non-equilibrium assay (sensitivity 10 pg/tube), as appropriate. Intra-assay variability was <5% and inter-assay variation was <7%.

Extraction from normal plasma.—10 ml plasma was acidified with 2 ml 1 mol/l hydrochloric acid and passed twice through SEP-PAK C18 (Waters Associates) cartridges washed in 30 ml methanol/water (80/20 v/v) and pre-equilibrated with 20 ml trifluoroacetic acid/water (0·1/99·9 v/v). KC was eluted with 10 ml methanol/water/TFA (79/20/1 v/v) and stored at −20°C after drying under vacuum until assay. The intra and inter assay variability was 10·5% and 13·5%, respectively; mean recovery of KC from plasma was 62%.

Immunoassay characteristics.—Plasma KC values for the direct and extraction assay were obtained from displacement curves for synthetic KC standards alone, or for added KC extracted from blank plasma, respectively (fig 1). The presence of up to 10 μg/tube of the following did not interfere with the assay: luteinising hormone releasing hormone, CT (human and salmon), corticotropin (ACTH) 1-24, ACTH 1-39, oxytocin, prolactin, leu-enkephalin, gastric inhibitory polypeptide, cholecystokinin-8, glucagon, motilin, vasoactive intestinal polypeptide, somatostatin, parathormone, insulin, gastrin-17, secretin, bombesin, gastrin-releasing peptide, neurotensin, PHI,[4] pro-opiomelanocortin, growth hormone.

Statistical Methods

Analysis of variance and paired and unpaired two-tailed Student's t-test were used after $\log_{10}$ transformation of data. Linear regression analysis was also performed on $\log_{10}$-transformed data.

Results

Normal volunteers.—Our assay demonstrates unequivocally the presence of immunoreactive KC in each of the 57 normal subjects. As with CT,[10] plasma KC concentrations were much higher in men (64 *vs* 26 ng/l) than in women, except in those women taking the combined oral contraceptive pill (62 ng/l) (see accompanying table, fig 2). 5

PLASMA KC IN MEN AND WOMEN AND WOMEN ON ORAL CONTRACEPTIVES

—	Men	Women	Women on OC
Geometric mean (ng/l)	63·5	25·8	61·5
Mean $\log_{10}$ value	1·803	1·411	1·789

Difference between mean $\log_{10}$ values	(male-female) 0·392* (t=10·514)
	(female-female on OC) −0·378* (t=8·786)
	(male-female on OC) 0·014 (t=0·385)

OC=oral contraceptives.
*p<0·001.

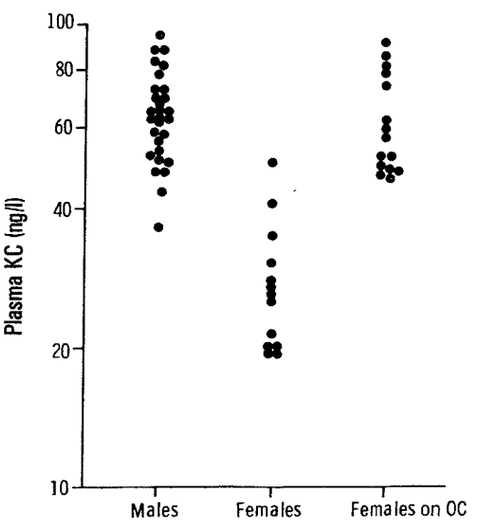

Fig 2—Plasma KC levels (ng/l) in males, females, and females on oral contraceptives.

min after calcium infusion, plasma KC and CT concentrations reached a peak of double their basal levels (fig 3).

Patients.—Plasma KC was markedly raised in 20 patients (range 0·58–486 μg/l) with medullary thyroid carcinoma. There was a close correlation between concentrations of KC and CT in both normal volunteers and patients (r=0·98, p<0·001).

Discussion

The major part of the human CT precursor structure was predicted from the partial nucleotide sequence of the precursor mRNA from medullary thyroid carcinoma, based on cloning and DNA sequence analysis of cDNA.[2] Recently, however, the structure of KC has been fully confirmed by formal isolation of the peptide from medullary carcinoma and structural determination by mass spectrometry (H. R. Morris, S. I. Girgis, T. R. Arnett, A. T. Etienne, M. Panico, and I. MacIntyre, unpublished). In further recent work we have localised KC in normal C-cells, by both immunofluorescent and electronmicroscopic techniques (A. Ali-Rachedi, I. M. Varndell, P. Facer, C. J. Hillyard, I. MacIntyre, and J. Polak, unpublished). Together with these studies, our new immunoassay findings leave little doubt that

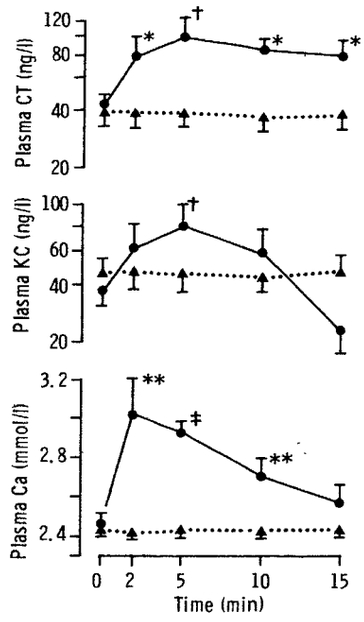

Fig 3—Plasma calcium (mmol/l), KC (ng/l), and CT (ng/l) (mean±SEM) after 1 min calcium (●—●) or saline (▲.....▲) infusions.

KC is a circulating hormonal peptide in normal subjects as well as in patients with medullary carcinoma. As might be expected, CT and KC circulate in approximately equimolar amounts (fig 4) and reflect equally changes in C-cell secretion. The position of KC and CT in their common precursor is shown in fig 5.

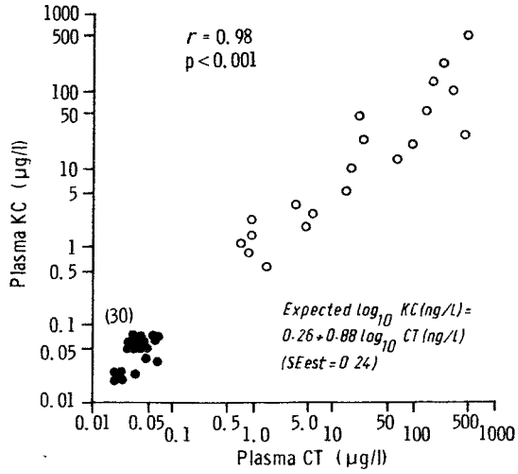

Fig 4—Plasma KC *vs* CT levels in normals (●) and patients with MCT (O).

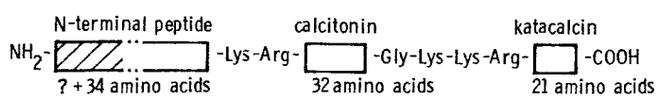

Fig 5—Position of CT and KC within their common precursor.[3]

Although KC is active in ng doses in the rat and in µg amounts in mouse calvariae organ culture, clinical studies of its effects in man are necessary before its physiological importance can be assessed. These studies will have to include not only healthy volunteers but also, where appropriate, patients with Paget's disease. It is only in these patients that appreciable plasma calcium changes are seen even after the administration of CT, because bone turnover is slow in normal adults. The raised plasma KC levels after calcium infusion suggest that, like CT, KC is normally involved in plasma calcium regulation. But the major physiological function of CT in adults may be concerned more with skeletal maintenance than with plasma calcium homoeostasis. Plasma CT is raised during growth, pregnancy, and lactation[11] when calcium demands are high. The effects of oestrogen replacement therapy in preventing bone loss[12] and of the contraceptive pill in increasing bone mass[13] have also been ascribed[14] to the associated increases in plasma CT. It now seems possible that since KC tends to change with CT (fig 3), all these postulated skeletal effects may depend on the presence of both hormones rather than on CT alone.

The hypocalcaemia which follows hypercalcaemic thyroid perfusion[15–17] or cautery[18] can no longer be attributed to CT alone. Measurement of plasma KC is likely to be useful in the diagnosis of medullary carcinoma of the thyroid and perhaps in the study of ectopic secretion of calcitonin. Finally, there may be a place for KC in the treatment of hypercalcaemia or Paget's disease where CT is at present the treatment of choice. In any event, it is clear that our findings will lead to substantial revision of current views of calcium regulation.

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