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# Regional Differences of Adenylate Cyclase Stimulation by Calcitonin and Calcitonin Gene-Related Peptide in the Human Kidney*

PIERANGELO GEPPETTI, ELISABETTA BALDI, STEFANO MANZINI, ELENA DEL BIANCO, CARLO ALBERTO MAGGI, ALESSANDRO NATALI, AND MASSIMO MANNELLI

*Institute of Internal Medicine and Clinical Pharmacology; Department of Clinical Physiopathology Endocrinology Unit, (E.B., M.M.), and the Institute of Urology (A.N.), University of Florence; the Department of Pharmacology, Malesci Pharmaceuticals (S.M.), and the Department of Pharmacology, Menarini Pharmaceuticals (C.A.M.), Florence, Italy*

**ABSTRACT.** Calcitonin (CT) gene-related peptide (CGRP)-like immunoreactivity was detected in both the cortex and medullo-papillary portion of human kidneys. The two forms of human CGRP as well as rat CGRP were capable of stimulating renal cortical adenylate cyclase activity in a concentration-related manner, with a half-maximally effective concentration ($EC_{50}$) similar to that of human CT and approximately 100–1000 times higher than that of salmon CT. However, in the medullo-papillary portion, in which both salmon CT and human CT were inactive, the two forms of human and rat CGRP increased adenylate cyclase activity by 100%, with $EC_{50}$ values ranging from 36 nmol/L to 1 μmol/L. In cortical membrane preparations the effect of CGRP was additive to that of salmon CT. We concluded that regional differences exist in the effect of CT and CGRP in human renal tissue and that in the medullo-papillary portion and possibly in the cortex, CGRP stimulates adenylate cyclase activity through a CT-independent mechanism. (*J Clin Endocrinol Metab* **69:** 491, 1989)

CALCITONIN (CT) gene-related peptide (CGRP) is a 37-amino acid peptide which is the product of alternative processing of the primary transcript of the CT gene (1, 2). CGRP has been detected by both immunohistochemistry and RIA in the thyroid, in neurons of the gastrointestinal tract, and within primary sensory neurons in rats and guinea pigs (3, 4). CGRP has various effects on cardiovascular, endocrine, and gastrointestinal tissues (5, 6). In particular, it is a powerful vasodilator (7). Two forms of CGRP have been found in the human central nervous system and peripheral tissues (8, 9).

Small but detectable amounts of CGRP-like immunoreactivity (CGRP-LI) have been found in the rat kidney (10). Sparse CGRP-immunoreactive nerve fibers have been demonstrated in rat (11), guinea pig (12), and human renal tissue (13). In certain tissues CGRP stimulates membrane adenylate cyclase (6, 14–16), and in some tissue this effect appears to be mediated by interaction with receptors for CT (17). We recently found that there are regional differences in CGRP-LI levels in various regions of rat renal tissue and that rat αCGRP (rCGRP), but not salmon CT (sCT) or rat CT (rCT), increased the accumulation of cAMP in the papillae of rat kidneys (18, 19).

Here we present evidence for CGRP-LI in human renal tissue and for the existence of regional differences in adenylate cyclase stimulation by CGRP and CT in this organ.

## Materials and Methods

### *Membrane preparation and tissue extraction*

Human kidneys were obtained at surgery from five patients (age range, 43–69 yr) undergoing nephrectomy for neoplastic lesions not involving the whole organ. The kidneys were placed in ice-cold 150 mmol/L NaCl immediately after nephrectomy, and all procedures were performed at 4 C. Fractions of tissue from either the cortical or medullo-papillary portion of the pole opposite to the tumor were used. The fractions used for peptide measurement were immediately frozen and kept at −80 C.

The cortical or medullo-papillary portions were homogenized in 3 mmol/L Tris-HCl and 1 mmol/L EDTA, pH 7.4 (1:5, wt/vol), using an Ultra-Turrax homogenizer. The homogenates were filtered through gauze and centrifuged at 20,000 × *g* for 15 min. The pellets were resuspended in 80 mmol/L Tris-HCl,

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Address all correspondence and requests for reprints to: Dr. P. Geppetti, Institute of Internal Medicine and Clinical Pharmacology, Viale Morgagni 85, 50134 Florence, Italy.
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pH 7.4, dispersed by means of a glass-Teflon homogenizer, and stored in liquid nitrogen until assayed. Protein content was determined using the Protein Assay Reagent (Pierce, IL), based on the method of Bradford (20), with BSA as standard.

For peptide measurement, the tissues were homogenized with an Ultra-Turrax homogenizer in 2 mol/L acetic acid (1:10, wt/vol), put in a boiling water bath for 10 min, cooled at 4 C for 10 min, and centrifuged at 20,000 × *g* for 20 min at 4 C. The supernatants were freeze-dried and stored at −20 C.

### *Adenylate cyclase activity*

Adenylate cyclase activity was measured in triplicate as the rate of conversion of [$^{14}$C]ATP to [$^{14}$C]cAMP, as previously described (21). The incubation medium (final volume, 100 μL) contained 80 mmol/L Tris-HCl (pH 7.4), 10 mmol/L theophylline, 1.5 mmol/L $MgSO_4$, 0.1 mmol/L ATP, 1 mmol/L cAMP, 0.58 μCi [$^{14}$C]ATP, 0.05 mmol/L GTP, 3.6 mg/mL creatine phosphate, 200 U/ml creatine kinase, and appropriate peptide concentrations. The reaction was started by adding membrane protein (0.15–0.2 mg/tube). The mixtures were incubated in a shaking water bath for 20 min at 37 C, and the reaction was stopped by adding 100 μL of a solution containing 2% sodium dodecyl sulfate, 40 mmol/L ATP, 1.4 mmol/L cAMP, 10 mmol/L Tris-HCl (pH 7.4), and 0.17 μCi [$^{3}$H]cAMP. [$^{14}$C]- and [$^{3}$H]cAMP were separated from [$^{14}$C]ATP by sequential chromatography according to the method of Solomon *et al.* (22). Each value was corrected for cAMP recovery (70–80%), as estimated from the recovered [$^{3}$H]cAMP added to the tubes, and the blank value was determined in the absence of membranes. Adenylate cyclase activity was expressed as picomoles of cAMP generated per min/mg protein. Enzyme activity was directly proportional to protein concentration over the range used in the assay.

Cortical membrane preparations were tested with 0.1 μmol/L PTH which increased the formation of cAMP by about 900%. Arginine vasopressin (0.1 μmol/L) was used for testing medullo-papillary samples, in which it enhanced adenylate activity by about 80%.

### *CGRP-LI RIA*

CGRP-LI was measured in duplicate, as described previously (23). Samples reconstituted in the assay buffer (0.1 mol/L phosphate buffer, pH 7.4, containing 150 mmol/L NaCl, 0.1 g/L $NaN_3$, and 1 g/L BSA) or human CGRP-II (CGRP-II) were incubated with anti-CGRP antiserum (raised in a rabbit against human CGRP-II) for 48 h at 4 C. [$^{125}$I]Iodohistidyl-human CGRP then was added and incubated for a further 24 h at 4 C. Separation of bound from free antigen was obtained by double antibody precipitation. The percent coefficient of variation was less than 10% for values between 20–300 pmol/L, and the lowest detectable concentration was 2 fmol/tube. The cross-reactivities of rCGRP and human CGRP-I (CGRP-I) were 100%, and those of sCT and human CT (hCT) were less than 0.01%.

### *Statistical analysis*

The results are given as the mean ± SE. Statistical analysis was performed by means of Student's *t* test for paired data or Dunnett's test when applicable. For calculation of the half-maximally effective concentration ($EC_{50}$), concentration-response curves were analyzed by the program Allfit (24). $P < 0.05$ was considered significant.

### *Reagents*

The reagents used were purchased from the indicated sources: sCT, hCT, rCGRP, CGRP-I, CGRP-II, and antihuman CGRP-II antiserum (RAS 6012) from Peninsula (Belmont, CA); PTH and arginine vasopressin from Sigma (St. Louis, MO); [$^{125}$I]CGRP; [$^{14}$C]ATP and [$^{3}$H]cAMP from Amersham (Little Chalfont, United Kingdom); and ATP, cAMP, creatine phosphate, and creatine kinase from Boeringher (Mannheim, West Germany).

## Results

### *CGRP-LI tissue levels*

CGRP-LI was detected in all samples analyzed. The concentration was slightly but not significantly higher in the medullo-papillary portion than in the cortex (Table 1). Serial dilutions of the samples gave responses in the RIA that paralleled those of the standard (data not shown).

### *Adenylate cyclase stimulation*

The mean basal adenylate cyclase activity in the cortical membrane preparations was 21.2 ± 1.4 pmol/mg protein·min (nine triplicate determinations from five membrane preparations). All peptides stimulated cortical adenylate cyclase activity in a concentration-dependent manner (Fig. 1). The maximal effect was caused by 0.1 μmol/L sCT (240 ± 31% of the basal activity; $EC_{50}$ = 1.9 ± 0.6 nmol/L). hCT was approximately 100 times less potent than sCT and induced half of the maximal effect produced by sCT. CGRP-I and rCGRP produced a greater maximal increase in enzyme activity than hCT, but both were less potent than the two forms of CT. CGRP-II was less potent, with a maximal effect (at 10

TABLE 1. CGRP-LI (femtomoles per g wet wt) in cortex and medullo-papillary portion of five human kidneys

| Patient no. | Cortex CGRP-L | Medullo-papillary portion CGRP-LI |
|---|---|---|
| 1 | 120 | 580 |
| 2 | 321 | 1543 |
| 3 | 527 | 761 |
| 4 | 302 | 274 |
| 5 | 223 | 278 |
| Mean ± SE | 296 ± 66 | 672 ± 181 |

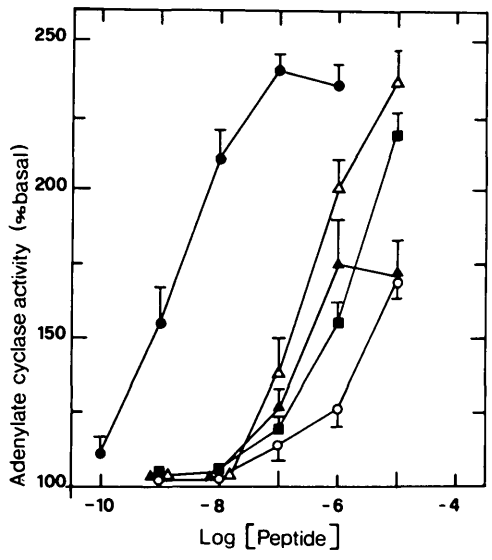


FIG. 1. Effect of sCT (●), hCT (▲), rCGRP (△), CGRP-I (■), and CGRP-II (○) on adenylate cyclase activity of cortical membrane preparations from five human kidneys. Each *point* represents the mean ± SE of the percent increase over basal activity (100%).

μmol/L) less than 50% of that produced by sCT (1 μmol/L; Fig. 1 and Table 2).

In the medullo-papillary portion basal adenylate cyclase activity was 46.1 ± 2.2 pmol/mg protein·min (nine triplicate experiments from five membrane preparations). In this region both sCT and hCT up to 10 μmol/L failed to stimulate adenylate cyclase activity. At this concentration the two forms of CT had only a small, not significant, effect (Fig. 2). However, CGRP-I, CGRP-II, or rCGRP raised adenylate cyclase activity by about 100% in a concentration-related manner (Fig. 2). rCGRP was again slightly more potent than either CGRP-I or CGRP-II (Fig. 2 and Table 2).

Incubation of cortical membrane preparations with the maximally effective concentrations of sCT (0.1 μmol/L) and CGRP-I (10 μmol/L) produced a significantly greater accumulation of cAMP ($P < 0.01$) than incubation with each peptide alone (Table 3).

## Discussion

Regional differences in the stimulation of adenylate cyclase by sCT have been found in the rat kidney (25). sCT was slightly more active in the cortex than in the outer medulla and almost inactive in the papilla. In the rabbit kidney, sCT stimulated adenylate cyclase activity in various regions of the cortex or outer medulla, but not in the inner medulla (26). We have confirmed that sCT stimulates adenylate cyclase in the cortex and medulla of rat kidney, but not in the papilla, and that only rCGRP acts in all three regions of the rat kidney (18, 19). In cortical membrane preparations of human kidney, sCT, hCT, rCGRP, and CGRP-I are all capable of producing concentration-dependent accumulation of cAMP, and sCT was the most potent compound, with an $EC_{50}$ 100–1000 times lower than those of hCT and the various CGRP forms. However, only CGRP was active in the medullo-papillary portion of human kidney, indicating the existence in this region of CGRP-sensitive, but not CT-sensitive, adenylate cyclase. CGRP-I increased significantly the maximal stimulation produced by sCT in cortical tissue, suggesting that at this level adenylate cyclase specifically activated by CGRP could be present. Since little is known about the enzyme(s) involved in CGRP metabolism and, accordingly, about compounds able to inhibit CGRP degradation, peptidase inhibitors were not used in our experiments. Thus, we cannot exclude the possibility that proteolytic activity of human renal tissue might play an important role in determining the relative potencies of the various forms of CGRP and CT.

Different results have been reported concerning adenylate cyclase stimulation by sCT in the human kidney. Experiments using a single kidney preparation failed to identify enzyme stimulation in any part of the kidney (27). In microdissected collagenase-treated human kidneys sCT increased adenylate cyclase activity in the thick ascending limb and distal convoluted tubule, while minor stimulation was found in the cortical and medullary collecting tubules (28). hCT increased the production of immunoreactive cAMP in both cortical and medullary membrane preparations, while sCT was active only in the cortex (29). The discrepancies between these findings may be ascribed to different methods of sample preparation and measurement of enzyme activity.

Certain effects exerted by CGRP in a variety of tissues

TABLE 2. Stimulation of adenylate cyclase activity in the cortex and medullo-papillary portion of human renal tissue (n = 5) by sCT, hCT, rCGRP, CGRP-I, and CGRP-II

| | sCT | hCT | rCGRP | CGRP-I | CGRP-II |
|---|---|---|---|---|---|
| Cortex | | | | | |
| $ED_{50}$ (nmol/L) | 1.9 ± 0.6 | 260 ± 20 | 350 ± 120 | 10,900 ± 190 | ND |
| % Maximum effect | 240 ± 31 | 175 ± 18 | 235 ± 19 | 219 ± 15 | 169 ± 21 |
| Medullopapillary portion | | | | | |
| $ED_{50}$ (nmol/L) | ND | ND | 36 ± 0.6 | 800 ± 20 | 1000 ± 150 |
| % Maximum effect | 108 ± 3.7 | 114 ± 9.3 | 190 ± 8 | 200 ± 9 | 204 ± 11 |

Values are the mean ± SE. % Maximum effect, Maximum effect as percentage of basal (100%). ND, Not determinable.

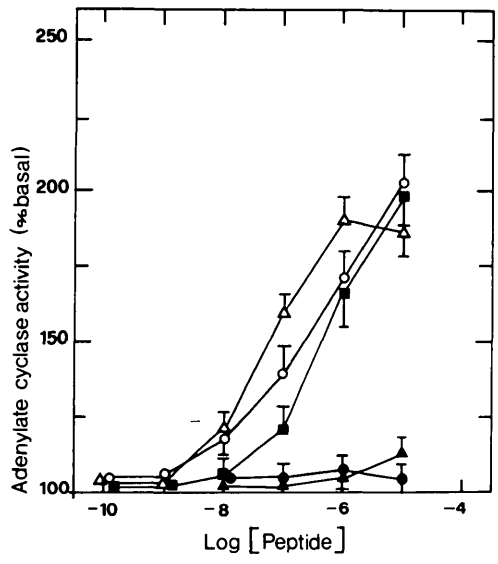


FIG. 2. Effects of sCT, hCT, rCGRP, CGRP-I, and CGRP-II on the adenylate cyclase activity of medullo-papillary membrane preparations from five human kidneys. For symbols, see Fig. 1. Each *point* represents the mean ± SE of the percent increase over basal activity (100%).

TABLE 3. Production of cAMP in cortical membrane preparations (n = 8) of human kidney by sCT and CGRP-I

| | pmol/mg protein·min | % of basal |
|---|---|---|
| Basal | 18.5 ± 1.1 | 100 |
| sCT (0.1 μmol/L) | 40.7 ± 2.1 | 220 |
| CGRP-I (10 μmol/L) | 37.5 ± 1.2 | 202 |
| sCT (0.1 μmol/L) and CGRP-I (10 μmol/L) | 50.4 ± 1.8[a] | 272 |

Values are the mean ± SE.
[a] $P < 0.01$ *vs.* both sCT and CGRP-I.

are thought to be mediated by cAMP (6, 14–16). However, a specific CGRP-sensitive adenylate cyclase was reported to be absent in the rat kidney, and CGRP action at this level was considered due to cross-reaction with CT receptors (17). The recent observation that rCGRP increased cAMP accumulation in the papillae of rat kidney, where sCT is inactive (18, 19), seems to rule out the possibility that adenylate cyclase stimulation by CGRP is mediated by CT receptors. Likewise, in the medullo-papillary portion of the human kidney CGRP seems to act through a CT-independent mechanism. The existence of a subclass of CGRP-binding sites, which exhibits high affinity for both CT and CGRP, has been described in discrete areas of the rat brain (30). However, in the human renal cortex we have not found CT-sensitive adenylate cyclase exhibiting high affinity for CGRP.

CGRP-immunoreactive nerve fibers in rat and guinea pig kidney were reduced after capsaicin pretreatment (13, 31). CGRP-LI of rat kidney was sensitive to both *in vivo* and *in vitro* administration of capsaicin (18, 19). These findings indirectly indicate that immunoreactive CGRP is contained within primary sensory neurons (see Ref. 32 for review). The origin of the CGRP-LI found in human renal tissue remains to be determined, although in this organ CGRP immunoreactivity has been localized only within nerve fibers (17).

CGRP infusion into rat renal arteries reduced the increase in perfusion pressure elicited by norepinephrine (18). CGRP infusion in normal anesthetized rats increased renal blood flow and reduced urinary sodium excretion (33); in humans it induced hypotension (34). CGRP administered iv increased PRA in humans (31). In this context, it is interesting to note that the estimated $EC_{50}$ values of certain recently described effects of CGRP, such as renin release and cAMP accumulation in cultured rat juxtaglomerular cells (100 nmol/L) (31) and adenylate cyclase stimulation in membrane preparation of rat papillae (19 nmol/L) (18), are similar to those found in this study.

In conclusion, effects in human renal tissue may be mediated by cAMP. CGRP immunoreactivity has been found within renal nerve fibers predominantly distributed around blood vessels (13), suggesting the vascular cells as a possible target of CGRP action. However, the renal structure(s) at which CGRP selectively induces cAMP accumulation is unknown.

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