Calcitonin gene-related peptide receptor antagonist human CGRP-(8—37)

TSUTOMU CHIBA, AKINORI YAMAGUCHI, TOSHIYUKI YAMATANI,
AKIRA NAKAMURA, TOMOYUKI MORISHITA, TETSUYA INUI,
MASAAKI FUKASE, TOSHIHARU NODA, AND TAKUO FUJITA
Third Department of Internal Medicine, Kobe University School of Medicine,
Kobe 650; and Toyo Jozo Research Laboratory, Shizuoka 632-1, Japan

CHIBA, TSUTOMU, AKINORI YAMAGUCHI, TOSHIYUKI YAMATANI, AKIRA NAKAMURA, TOMOYUKI MORISHITA, TETSUYA INUI, MASAAKI FUKASE, TOSHIHARU NODA, AND TAKUO FUJITA. *Calcitonin gene-related peptide receptor antagonist human CGRP-(8—37).* Am. J. Physiol. 256 (Endocrinol. Metab. 19): E331-E335, 1989.—From this study, we predicted that the human calcitonin gene-related peptide (hCGRP) fragment hCGRP-(8—37) would be a selective antagonist for CGRP receptors but an agonist for calcitonin (CT) receptors. In rat liver plasma membrane, where CGRP receptors predominate and CT appears to act through these receptors, hCGRP-(8—37) dose dependently displaced ^{125}I-[Tyr^0]rat CGRP binding. However, hCGRP-(8—37) had no effect on adenylate cyclase activity in liver plasma membrane. Furthermore, hCGRP-(8—37) inhibited adenylate cyclase activation induced not only by hCGRP but also by hCT. On the other hand, in LLC-PK$_1$ cells, where calcitonin receptors are abundant and CGRP appears to act via these receptors, the bindings of ^{125}I-[Tyr^0]rat CGRP and ^{125}I-hCT were both inhibited by hCGRP-(8—37). In contrast to liver membranes, interaction of hCGRP-(8—37) with these receptors led to stimulation of adenosine 3', 5'-cyclic monophosphate (cAMP) production in LLC-PK$_1$ cells, and moreover, this fragment did not inhibit the increased production of cAMP induced not only by hCT but also by hCGRP. Thus hCGRP-(8—37) appears to be a useful tool for determining whether the action of CGRP as well as that of CT is mediated via specific CGRP receptors or CT receptors.

LLC-PK$_1$ cells; calcitonin gene

ALTERNATIVE TISSUE specific processing of initial mRNA transcripts from the calcitonin gene generates two distinct peptides: calcitonin and calcitonin gene-related peptide (CGRP) (1, 16). Although these two peptides have little sequence homology, calcitonin and CGRP possess similar structural characteristics, i.e., similar sizes, a disulfide bridge at the NH$_2$-terminal portion, and an amidated carboxy-terminal end. On the other hand, previous pharmacological studies have demonstrated the presence of distinct receptors for calcitonin and for CGRP in various tissues and cells (5, 7, 11, 12, 19, 20).

Moreover, recent receptor cross-linking studies followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis have revealed the existence of specific CGRP receptors, which are distinct from calcitonin receptors (2, 13), with differing molecular sizes in various tissues (3, 4, 7). Because of the structural similarities mentioned above, however, these two peptides have also been shown to cross-react with each other at the levels of their receptors (6, 12, 19, 20). Furthermore, it has also been reported that specific receptors for both calcitonin and CGRP appear to coexist within the same tissue, such as brain (6). As a result, it is extremely difficult to elucidate the direct actions of these peptides on their respective receptors. Previously, however, we have demonstrated that rat liver plasma membrane exclusively possesses CGRP receptors and that calcitonin appears to interact with these receptors (20). On the other hand, Wohlwend et al. (19) have reported that, in LLC-PK$_1$ pig kidney cells, calcitonin receptors predominate and that the stimulation of adenylate cyclase activity by CGRP is probably mediated by these receptors. Therefore, by using both LLC-PK$_1$ cells and rat liver plasma membrane, it should be possible to characterize specific CGRP receptors and calcitonin receptors separately.

Recently, we have shown that a human CGRP fragment, human CGRP-(8—37), binds to CGRP receptors in rat liver plasma membrane (20). In this study, by examining the effects on not only CGRP receptors in liver plasma membrane but also calcitonin receptors on LLC-PK$_1$ cells, we found that this fragment, human CGRP-(8—37), is a specific antagonist for CGRP receptors but not for calcitonin receptors, raising the possibility of being able to clarify whether the CGRP or the calcitonin receptor is responsible for the action of either of these two peptides under various conditions using this CGRP antagonist.

MATERIALS AND METHODS

Peptides and radioligands. Synthetic [Tyr^0]rat CGRP and human calcitonin were purchased from Peninsula

(Belmont, CA). Human CGRP and human CGRP-(8—37) were synthesized using an automatic solid-phase synthesizer (430-A Peptide Synthesizer, Applied Biosystems. ^{125}I-[Tyr^0]rat CGRP (2,000 Ci/mmol) was prepared as previously described (14), and ^{125}I-human calcitonin (2,000 Ci/mmol) was supplied by Amersham (Buckinghamshire, UK). Human calcitonin was radioiodinated by the chloramine-T method, and the methionine-oxidized form of ^{125}I-human calcitonin was removed by reverse-phase high-performance liquid chromatography (HPLC). An adenosine 3′,5′-cyclic monophosphate (cAMP) assay kit was obtained from Yamasa Shoyu (Choshi, Japan) (17).

Preparation of rat liver plasma membranes, membrane receptor binding study, and membrane adenylate cyclase assay. Rat liver plasma membranes were prepared from adult male Sprague-Dawley rats by the method of Pilkis et al. (15) with slight modification, as described previously (20). The fraction collected at the 42.5–48.2% sucrose interface was removed and washed twice with 10 mM N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES) buffer (pH 7.4) containing 1 mg/ml bacitracin. The final pellet was resuspended in 10 mM HEPES buffer (pH 7.4) containing 120 mM NaCl, 4.7 mM KCl, 5 mM MgCl$_2$, 1 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), 1 mg/ml bacitracin, and 5 mg/ml bovine serum albumin (BSA). One-half milliliter of this membrane suspension (200 μg of tissue protein) containing ^{125}I-[Tyr^0]rat CGRP (25 pM) was incubated at 24°C for 60 min with or without various unlabeled peptides. Bound and free hormone were separated by centrifugation and radioactivity bound to the pellets was counted by an autogamma counter. Nonspecific binding was assessed as the fraction of label that remained bound in the presence of 10^{-6} M [Tyr^0]rat CGRP.

The adenylate cyclase activity in the membranes was determined by following the synthesis of cAMP from nonradioactive ATP, as described by Katada and Ui (8). Membranes (10 μg of protein) were incubated for 10 min at 37°C in 100 μl of tris(hydroxymethyl)aminomethane (Tris) buffer (25 mM, pH 7.6) containing 1 mM EGTA, 1 mg/ml of BSA, 100 mM NaCl, 100 μM GTP, 1 mM ATP, 5 mM MgCl$_2$, 1 mM 3-isobutyl-1-methylxanthine (IBMX), 5 mM phosphocreatine, 50 U/ml of creatine phosphokinase, and 1 mM aprotinin with or without various stimulants. The reactions were terminated by adding 0.33 M ZnSO$_4$(40 μl) and 0.5 M NaCO$_3$ (40 μl), and after being left to stand for 1 h at 4°C, samples were centrifuged and cAMP levels in the supernatant were measured by a sensitive radioimmunoassay (RIA) (17). The adenylate cyclase activity was expressed as picomoles of cAMP synthesized by 1 mg of membrane protein during 1 min of incubation.

LLC-PK$_1$ cell culture and study of receptor binding and cAMP production in these cells. LLC-PK$_1$ pig kidney cells (ATCC, CRL 1392) (19) were grown at 37°C in a 1:1 mixture of Ham's F12-Dulbecco's modified Eagle's medium with 10% fetal calf serum (FCS) under humidified 95% O$_2$-5% CO$_2$ atmosphere. For the experiments, the cells were washed with phosphate-buffered saline, dis-
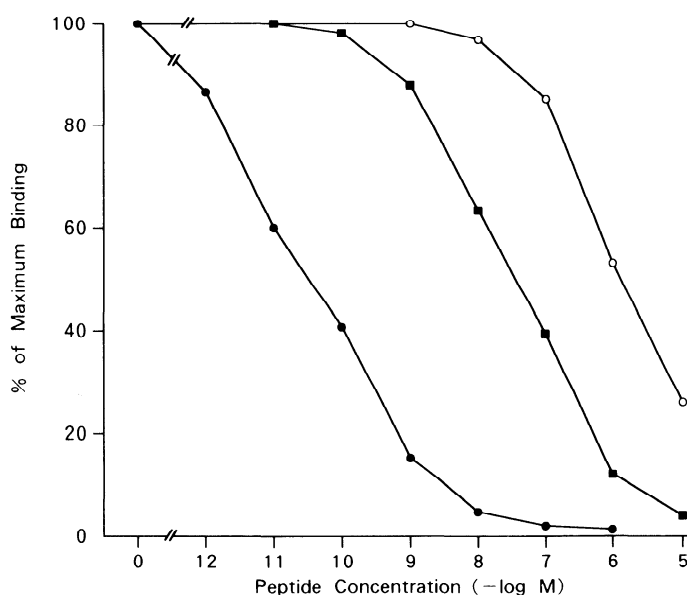

FIG. 1. Dose-response inhibition of ^{125}I-[Tyr^0]-rat calcitonin gene-related peptide (CGRP) binding to rat liver plasma membranes by human CGRP (●), human CGRP-(8—37) (■), and human calcitonin (○). Rat liver plasma membranes were incubated for 60 min at 24°C with 25 pM ^{125}I-[Tyr^0]rat CGRP plus peptides as indicated. Total and specific bindings were 11.4 ± 1.8 and 10.3 ± 1.4% of total radioactivity added, respectively. Data are expressed as percentages of maximal specific binding in absence of unlabeled peptides. Values are means of 6 separate experiments differing by <5%. Each experiment was done in duplicate.
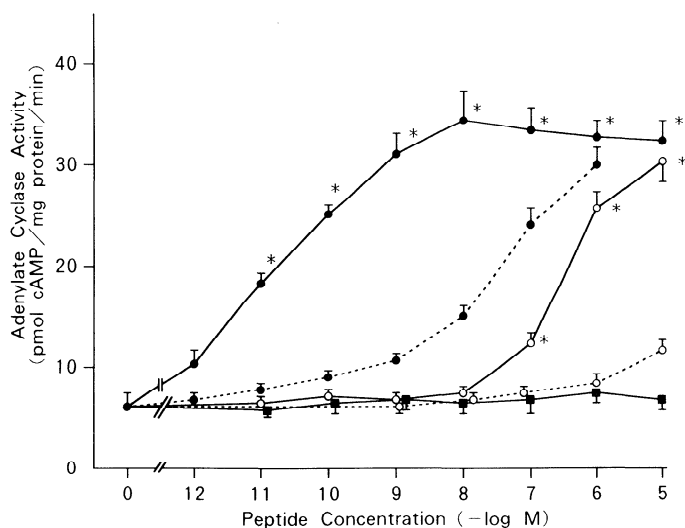

FIG. 2. Effect of human calcitonin gene-related peptide (CGRP)-(8—37) on human CGRP-stimulated as well as human calcitonin-stimulated adenylate cyclase activation in rat liver plasma membrane. Rat liver plasma membranes were incubated either with increasing concentrations of human CGRP (●——●), human CGRP-(8—37) (■——■), or human calcitonin (○——○) alone or in combination with human CGRP plus human CGRP-(8—37) (10^{-5} M; ●- - -●) or human calcitonin plus human CGRP-(8—37) (10^{-5} M; ○- - -○) for 10 min at 37°C. Each value represents mean ± SE of 5 separate experiments. Each experiment was done in duplicate. Statistical analysis was done by analysis of variance in conjunction with Duncan's new multiple range test. *$P < 0.01$ vs. control value.

persed with 0.5% trypsin and 1 mM EDTA, and preincubated in HEPES-buffered Krebs-Ringer bicarbonate buffer with 1 mg/ml BSA and 2.5 mM glucose (HKRBG), pH 7.4, for 1 h. The binding study was carried out by
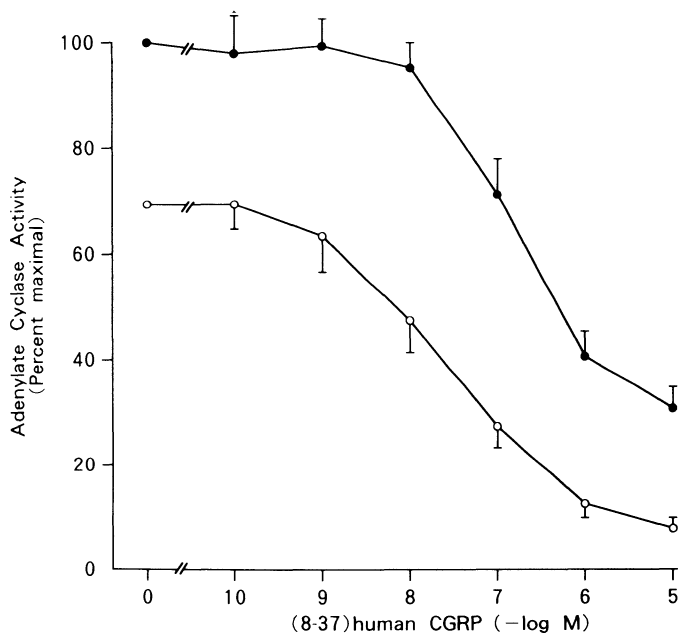
FIG. 3. Effect of increasing concentration of human calcitonin gene-related peptide (CGRP)-(8—37) on stimulation of adenylate cyclase activity in rat liver plasma membrane induced by human CGRP (10^{-8} M; ●) or human calcitonin (10^{-6} M; ○). Rat liver plasma membranes were incubated with either human CGRP (10^{-7} M) or human calcitonin (10^{-6} M) in the presence or absence (control) of increasing concentrations of human CGRP-(8—37) for 10 min at 37°C. Data are expressed as percentages of the increase in adenylate cyclase activity (from control) induced by a maximally effective concentration of human CGRP (10^{-8} M). Results are means ± SE of 5 separate experiments. Each experiment was done in duplicate.

TABLE 1. *Effect of human CGRP-(8—37) (10^{-5} M) on adenylate cyclase activation induced by epinephrine or glucagon in rat liver plasma membrane*

Agents	Adenylate Cyclase Activity, pmol cAMP·mg protein^{-1}·min^{-1}	
	Alone	With human CGRP-(8—37)
Control	6.2±0.5	5.8±0.4
Epinephrine (10^{-6} M)	23.1±2.0	23.5±1.8
Glucagon (10^{-8} M)	56.8±4.6	57.2±5.0

Values are means ± SE of 5 separate experiments. Rat liver plasma membranes were incubated with or without epinephrine (10^{-6} M) or glucagon (10^{-8} M) in presence or absence of human CGRP-(8—37) (10^{-5} M) for 10 min at 37°C. Each experiment was done in duplicate. Student's t test was used for statistical analysis. See text for definition of abbreviations.

incubating the cells (2 × 10^6/0.5 ml) with ^{125}I-[Tyr^0]rat CGRP (50 pM) or ^{125}I-human calcitonin (25 pM) in the presence or absence of various unlabeled peptides at 37°C for 60 min. Cells were separated by centrifugation, and the radioactivity bound to the cells was counted. Specific binding was defined as total binding minus nonspecific binding in the presence of 10^{-6} M unlabeled human calcitonin.

In the cAMP production study, the cells (10^6) were incubated in 1 ml HKRBG with or without various agents in the presence of IBMX (10^{-4} M) at 37°C. After 15 min, the incubations were terminated by adding 1 ml of ice-cold 12% trichloroacetic acid, centrifuging the mixture, and washing the supernatant three times with water-saturated diethylether. cAMP levels were determined by a specific RIA (17).

RESULTS

The specific binding of ^{125}I-[Tyr^0]-rat CGRP to rat liver plasma membrane was displaced by human CGRP, human CGRP-(8—37) and by human calcitonin in a dose-dependent manner with relative affinities as follows: human CGRP > human CGRP-(8—37) > human calcitonin (Fig. 1). Of these three peptides, both human CGRP and human calcitonin significantly stimulated adenylate cyclase activation in a dose-dependent fashion in rat liver membrane, but human CGRP was clearly more potent than human calcitonin (Fig. 2). Furthermore, the doses of these peptides necessary for producing a half-maximal effect on adenylate cyclase activation (human CGRP, 21.5 pM; human calcitonin, 0.46 μM) were comparable to those producing half-maximal inhibition of label binding to the membrane (32.1 pM and 1.32 μM, respectively). In contrast, human CGRP-(8—37) did not have any effect on adenylate cyclase activity up to 10^{-5} M, and moreover, in the presence of 10^{-5} M human CGRP-(8—37), the dose-response curve for adenylate cyclase activation induced not only by human CGRP but also by human calcitonin was shifted to the right (Fig. 2). Indeed, the inhibitory effect of human CGRP-(8—37) on human CGRP-induced as well as human calcitonin-induced adenylate cyclase activation was dose-dependent (Fig. 3). However, human CGRP-(8—37) (10^{-5} M) had no effect on either epinephrine (10^{-6} M)- or glucagon (10^{-7} M)-induced activation of adenylate cyclase (Table 1).

On the other hand, all of human CGRP, human CGRP-(8—37) and human calcitonin displaced the specific binding of ^{125}I-[Tyr^0]-rat CGRP as well as that of ^{125}I-human calcitonin to LLC-PK$_1$ cells in a dose-dependent fashion (Fig. 4). The relative potencies of these peptides for the displacement of ^{125}I-[Tyr^0]rat CGRP and ^{125}I-human calcitonin were similar, and in contrast to the result obtained with rat liver plasma membrane, human calcitonin was most potent followed by human CGRP and then by human CGRP-(8—37) (Fig. 4). In striking contrast to the data obtained with rat liver plasma membrane, it was found that in addition to human CGRP and human calcitonin, human CGRP-(8—37) also evoked a significant and dose-dependent increase in cAMP production in LLC-PK$_1$ cells (Fig. 5). Furthermore, human CGRP-(8—37) (10^{-5} M) had no effect on either human CGRP- or human calcitonin-induced adenylate cyclase activation (Fig. 5). The relative potencies of these peptides in stimulating cAMP production were similar to those in displacing the label bindings from LLC-PK$_1$ cells.

DISCUSSION

In the previous report, we identified specific binding sites for CGRP in rat liver plasma membrane and showed that various kinds of calcitonin interacted with these CGRP receptors (20). The present study demonstrated that human CGRP as well as human calcitonin induced
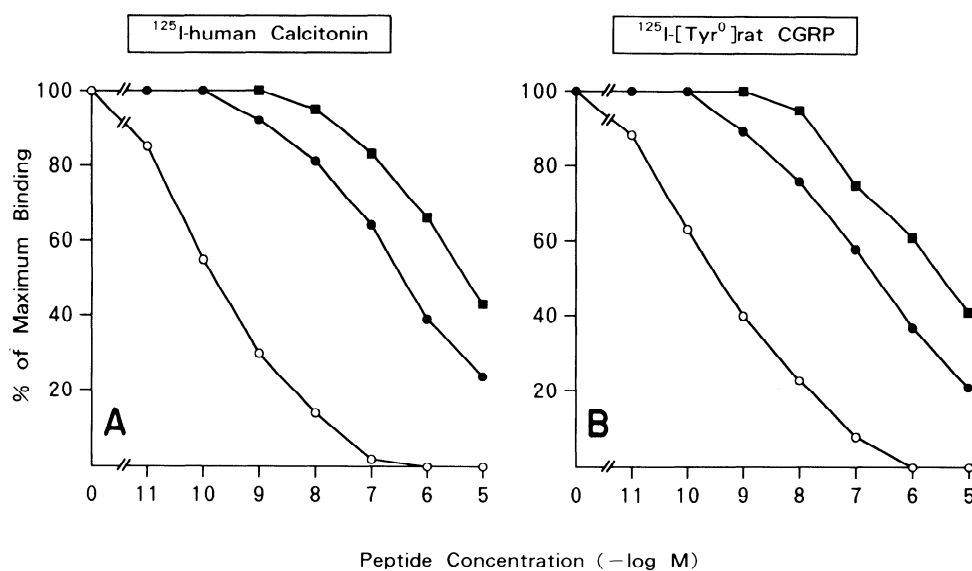
FIG. 4. Dose-response inhibition of ^{125}I-human calcitonin (A) or ^{125}I-[Tyr^0] rat calcitonin gene-related peptide (CGRP; B) binding to LLC-PK$_1$ cells by human CGRP (●), human CGRP-(8—37) (■), and human calcitonin (○). LLC-PK$_1$ cells were incubated for 60 min at 37°C with either 25 pM ^{125}I-human calcitonin or 50 pM ^{125}I-[Tyr^0]rat CGRP plus peptides indicated. Total and specific bindings of ^{125}I-human calcitonin were 14.5 ± 1.1 and 12.6 ± 1.0% of total radioactivity added, respectively, and those of ^{125}I-[Tyr^0]rat CGRP were 2.7 ± 0.4 and 2.4 ± 0.3%, respectively. Data are expressed as percentages of maximal specific binding in absence of unlabeled peptides. Values are means of 6 separate experiments differing by <6%. Each experiment was done in triplicate.
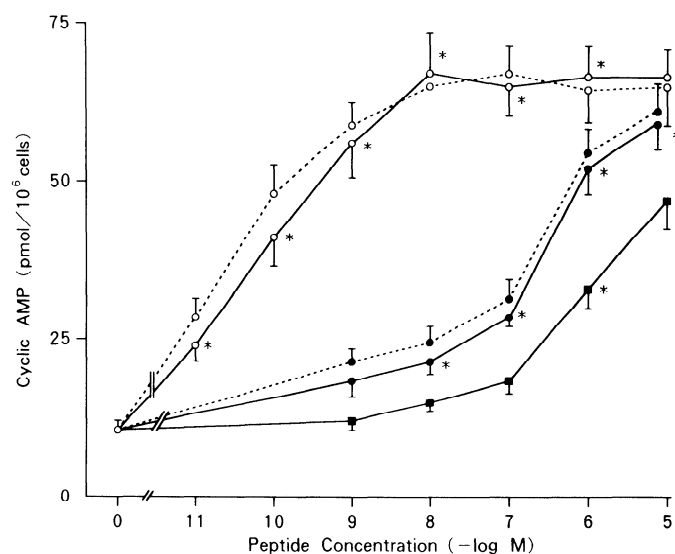

FIG. 5. Effect of human calcitonin gene-related peptide (CGRP)-(8—37) on human CGRP-induced as well as calcitonin-induced increase in cAMP production in LLC-PK$_1$ cells. LLC-PK$_1$ cells were incubated with increasing concentrations of human CGRP (●——●), human CGRP-(8—37) (■——■), or human calcitonin (○——○) alone or in combination with human CGRP plus human CGRP-(8—37) (10^{-5} M) (●– – –●) or human calcitonin plus human CGRP-(8—37) (10^{-5} M; ○– – –○). Each value represents mean ± SE of 5 experiments. Each experiment was done in duplicate. For statistical analysis, data were tested by analysis of variance in conjunction with Duncan's new multiple range test. *P < 0.01 vs. control value.

a dose-dependent activation of adenylate cyclase in rat liver plasma membrane. Thus, it appears that receptors for CGRP in rat liver plasma membrane are linked to the adenylate cyclase-cAMP system. However, in contrast to the effects of human CGRP and human calcitonin and despite its ability to bind to CGRP receptors, the present data clearly demonstrated that a human CGRP fragment, human CGRP-(8—37), had no effect on adenylate cyclase activity in rat liver plasma membrane and that, moreover, it significantly inhibited the activation of adenylate cyclase induced not only by human CGRP but also by human calcitonin. Thus it is strongly suggested that human CGRP-(8—37) acts as an

antagonist for the peptides that bind to CGRP receptors in rat liver plasma membrane.

On the other hand, LLC-PK$_1$ pig kidney cells have been reported to possess specific receptors for calcitonin, and CGRP appears to exert its action on these cells through the calcitonin receptors (19). In the present experiment, human calcitonin was more potent than human CGRP not only in displacing the specific bindings of ^{125}I-[Tyr^0]rat CGRP and ^{125}I-human calcitonin but also in activating adenylate cyclase in LLC-PK$_1$ cells, thus confirming previous reports (19). We, therefore, examined whether this CGRP receptor antagonist, human CGRP-(8—37), also antagonizes the actions of human CGRP and human calcitonin on calcitonin receptors in LLC-PK$_1$ cells, and it was found that this fragment not only stimulated adenylate cyclase activation but also had no inhibitory effect on adenylate cyclase activation induced by either human CGRP or human calcitonin. Thus it is evident that this CGRP receptor antagonist, human CGRP-(8—37), acts as an agonist for calcitonin receptors in LLC-PK$_1$ cells.

In a previous report of a study using rat liver plasma membranes, we suggested that the COOH-terminal portion of CGRP is essential for binding to its receptors, since an NH$_2$-terminal fragment of human CGRP could not bind to CGRP receptors, whereas COOH-terminal fragments were able to displace label binding (20). The present data add new evidence to support the fact that in spite of their ability to bind to CGRP receptors, COOH-terminal fragments of CGRP lacking a ring structure with a disulfide bond may not be sufficient to induce a subsequent intracellular signal transduction event, i.e., adenylate cyclase activation. Of particular interest in this study, however, was that this COOH-terminal fragment of human CGRP was able to promote adenylate cyclase activation, probably through specific calcitonin receptors in LLC-PK$_1$ cells. This is rather surprising, since this COOH-terminal portion of human CGRP has only slight amino acid sequence homology with human calcitonin. Goltzman (5), on the other hand, has reported that various modifications of the NH$_2$-terminal portion

of the calcitonin molecule including the disulfide bridge did not result in substantial reduction of its capacity to inhibit ^{125}I-salmon calcitonin binding and to stimulate adenylate cyclase in rabbit renal membranes. In agreement with his data, we found that the potency of human CGRP-(8—37), a COOH-terminal fragment of human CGRP, both for the binding and the stimulation of adenylate cyclase in LLC-PK$_1$ cells was not substantially different from that of human CGRP. Taken together, the COOH-terminal portion of the calcitonin molecule appears to be more important than the NH$_2$-terminal portion both in binding to its receptor and in stimulating adenylate cyclase, although the calcitonin receptor may have a broad specificity in recognizing the COOH-terminal portions of binding agonists.

Recent studies have revealed distinct localization of immunoreactive CGRP and calcitonin (14, 18) as well as localization of specific receptors for these peptides (5, 7, 11, 19, 20). In the brain, however, specific receptors appear to exist for both CGRP and calcitonin (6) and these peptides are known to cross-react with each other on their receptors (12, 19, 20). Thus, although it has been reported that intracerebroventricular administration of CGRP and calcitonin often exerts similar effects such as inhibition of gastric acid secretion (10) and reduction of food intake (9), it is still uncertain whether the actions of these peptides are mediated through calcitonin receptors or CGRP receptors. It is, therefore, tempting to predict that this newly discovered CGRP antagonist, human CGRP-(8—37), could be a useful tool for precise characterization of the actions of CGRP and calcitonin at the levels of their respective receptors.

Address for reprint requests: T. Chiba, Third Dept. of Internal Medicine, Kobe University School of Medicine, Kusunoki-cho 7-chome, Chuo-ku, Kobe 650, Japan.

REFERENCES

1. AMARA, S. G., V. JONAS, H. G. ROSENFELD, S. E. ONG, AND R. M. EVANS. Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products. *Nature Lond.* 298: 240–244, 1982.
2. BOUIZAR, Z., M. FOUCHEREAU-PERON, J. TABOULET, M. S. MOUKHTAR, AND G. GILHAUD. Purification and characterization of calcitonin receptors in rat kidney membranes by covalent cross-linking techniques. *Eur. J. Biochem.* 155: 141–147, 1986.
3. DOTTTI-SIGRIST, S., W. BRON, AND J. A FISCHER. Identification of a receptor for calcitonin gene-related peptide I and II in human cerebellum. *Biochem. Biophys. Res. Commun.* 151: 1081–1087, 1988.
4. FOORD, S. M., AND R. K. CRAIG. Isolation and characterization of a human calcitonin-gene-related-peptide receptor. *Eur. J. Biochem.* 170: 373–379, 1987.
5. GOLTZMAN, D. Examination of interspecies differences in renal and skeletal receptor binding and adenylate cyclase stimulation with human calcitonin. *Endocrinology* 106: 510–518, 1980.
6. GOLTZMAN, D., AND J. MITCHELL. Interaction of calcitonin and calcitonin gene-related peptide at receptor sites in target tissues. *Science Wash. DC* 227: 1343–1345, 1985.
7. HIRATA, Y., Y. TAKAGI, S. TAKATA, Y. FUKUDA, H. YOSHIDA, AND T. FUJITA. Calcitonin gene-related peptide receptor in cultured vascular smooth muscle and endothelial cells. *Biochem. Biophys. Res. Commun.* 151: 1113–1121, 1988.
8. KATADA, T., AND M. UI. Islet-activating protein: a modifier of receptor-mediated regulation of rat islet adenylate cyclase. *J. Biol. Chem.* 256: 8310–8317, 1981.
9. KRAHN, D. D., B. A. GOSNELL, A. S. LEVINE, AND J. E. MORLEY. Effects of calcitonin gene-related peptide on feeding in the rat. *Peptides Fayetteville* 5: 861–864, 1984.
10. LENZ, H. J., J. E. RIVIER, AND M. R. BROWN. Biological actions of human and rat calcitonin and calcitonin gene-related peptide. *Regul. Pept.* 12: 81–89, 1985.
11. MARSHALL, I., S. J. AL-KAZWINI, J. J. HOLMAN, AND R. K. CRAIG. Human and rat α-CGRP but not calcitonin cause mesenteric vasodilation in rats. *Eur. J. Pharmacol.* 123: 217–222, 1986.
12. MICHELANGELI, V. P., D. M. FINDLAY, A. FLETCHER, AND T. J. MARTIN. Calcitonin gene-related peptide (CGRP) acts independently of calcitonin on cyclic AMP formation in clonal osteogenic sarcoma cells (UMR 106-01). *Calcif. Tissue Int.* 39: 44–48, 1986.
13. MOSELEY, J. M., D. M. FINDLEY, T. J. MARTIN, AND J. J. GORMAN. Covalent cross-linking of a photoactive derivative of calcitonin to human breast cancer cell receptors. *J. Biol. Chem.* 257: 5846–5851, 1982.
14. OKIMURA, Y., K. CHIHARA, H. ABE, T. KITA, Y. KASHIO, M. SATO, AND T. FUJITA. Calcitonin gene-related peptide-like immunoreactivity in the central nervous system and peripheral organs of rats. *Regul. Pept.* 17: 327–337, 1987.
15. PILKIS, S. J., J. H. EXTON, A. ROGER, R. A. JOHNSON, AND C. R. PARK. Effects of glucagon on cyclic AMP and carbohydrate metabolism in livers from diabetic rats. *Biochim. Biophys. Acta* 343: 250–267, 1974.
16. ROSENFELD, M. G., J.-J. MERMOD, S. G. AMARA, L. W. SWANSON, D. E. SAWACHENKO, J. RIVIER, AND W. E. VALE. Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific RNA processing. *Nature Lond.* 304: 120–135, 1983.
17. SATO, T., K. SAITO, J. KAKEZAWA, AND H. YOSHIDA. Urinary excretion of cyclic nucleotides and principal electrolytes in healthy humans of different ages. *Clin. Chim. Acta* 110: 215–220, 1981.
18. SILVA, D. L., AND K. L. BECKER. Immunoreactive calcitonin in extrathyroidal tissues. In: *Calcitonin 1980.* Amsterdam: Exerpta Med., 1981, p. 144–153.
19. WOHLWEND, A., K. MALMSTROM, H. HENKE, H. MURER, J.-D. VASSALLI, AND J. A. FISCHER. Calcitonin and calcitonin gene-related peptide interact with the same receptor in cultured LLC-PK$_1$ cells. *Biochem. Biophys. Res. Commun.* 131: 537–542, 1985.
20. YAMAGUCHI, A., T. CHIBA, Y. OKIMURA, T. YAMATANI, T. MORISHITA, A. NAKAMURA, T. INUI, T. NODA, AND T. FUJITA. Receptors for calcitonin gene-related peptide on the rat liver plasma membranes. *Biochem. Biophys. Res. Commun.* 152: 376–382, 1988.