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GASTRIN/CCK-B RECEPTORS ON BRAIN, GASTRIC PARIETAL CELLS AND ECL CARCINOID TUMOR OF *MASTOMYS NATALENSIS*

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INDEXING WORDS

gastrin/CCK-B receptor; ECL carcinoid tumor; brain; gastric parietal cell

SYNOPSIS

Cholecystokinin (CCK)-8 and human gastrin I (gastrin) were found to be almost equipotent in displacing the specific binding of not only ^{125}I -CCK-8 but also ^{125}I-gastrin to both ECL carcinoid tumor and pareital cell membranes of *Mastomys natalensis*. These binding specificities of the native gastrin receptors for CCK-8 and gastrin were identical to that of the cloned gastrin receptor from the ECL carcinoid tumor expressed on CHO-K1 cells. In contrast, CCK-8 was approximately 10-20 times as potent as gastrin in inhibiting the binding of the two radioligands to the *Mastomys* brain membrane. However, the nucleotide sequences of RT-PCR products of mRNAs extracted from not only the isolated parietal cells but also the brain were identical to that of the gastrin receptor cDNA from the ECL carcinoid tumor. Thus, the gastrin receptors on the parietal cells and the brain appear to be encoded by the same gene as those on the ECL carcinoid tumor in *Mastomys*. The reason for the difference in binding characteristics between the cloned gastrin receptor and the native gastrin/CCK-B receptor in the brain remains to be elucidated.

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Abbreviations: ECL cell, enterochromaffin-like cell; CCK, cholecystokinin(sulfated form); BSA, bovine serum albumin; HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; EGTA, ethylene glycol-bis(b-aminoethylether)-N,N,N',N'-tetraacetic acid; RT-PCR, reverse transcriptase-polymerase chain reaction.

INTRODUCTION

Gastrin and cholecystokinin (CCK) play important physiological roles not only in the gastrointestinal tract but also in the central nervous system. Several lines of evedence suggest the existence of at least three distinct types of gastrin/CCK receptor. The CCK-A receptors possess high affinity for sulfated CCK-8 (CCK-8) and low affinity for human gastrin I and are present predominantly in the exocrine pancreas and gallbladder,[15,17] whereas CCK-B receptors have only a 10-fold higher affinity for CCK-8 than human gastrin I and are present in the brain.[13,14] On the other hand, the gastrin-type (gastrin) receptors have approximately equal affinity for CCK-8 and human gastrin I and are present in the fundic mucosa.[9,16] Indeed, Soll *et al.* [16] have demonstrated that CCK-8 and human gastrin I are equally potent not only in enhancing ^{14}C-aminopyrine uptake but also in displacing the binding of ^{125}I-human gastrin I from isolated canine parietal cells. In addition, we have identified specific gastrin receptors in the ECL carcinoid tumor of the fundic gland of *Mastomys natalensis,* which have similar binding characteristics to the gastin receptors on canine parietal cells.[4]

Recently, Kopen *et al.* [10] have cloned gastrin receptor cDNA from isolated canine parietal cells, and we have cloned a cDNA encoding the gastrin receptor from the ECL carcinoid tumor of *Mastomys* .[12] Furthermore, Wank *et al.*[19] have cloned a rat brain CCK-B receptor cDNA. Interestingly, the deduced amnino acid sequences of these three receptor cDNAs are highly homologous though not identical. Indeed, the ECL tumor gastrin receptor has 85.9% and 93.1% amino acid sequence homology with the canine parietal cell gastrin receptor and rat brain CCK-B receptor, respectively. However, because these three receptor cDNAs have been cloned from different species, it is still unclear whether gastrin/CCK-B receptors in the brain, gastric parietal cells and ECL cells are identical. Here, we report that in *Mastomys,* the nucleotide sequences of RT-PCR products of mRNAs extracted from both the brain and the gastic parietal cells are identical to that of the gastrin receptor cDNA from the ECL carcinoid tumor.

MATERIALS AND METHODS

Isolation of Mastomys parietal cells:

Mucosal cells were dispersed from stripped fundus of *Mastomys natalensis* by sequential exposure to collagenase and EDTA.[3] Isolated cells were enriched by a Beckman elutriator rotor and collected at 2,000 rpm between flow rates of 80 and 120 ml/min. Parietal cells were further enriched to 95% by Percoll density gradient (70%) centrifugation.

Crude membrane preparation and ^{125}I-CCK-8 and ^{125}I-human gastin I binding experiments:

Preparation of the *Mastomys* ECL carcinoid tumor membrane and the isolated parietal cell membrane was performed as described previously.[20] *Mastomys* brain membrane was prepared

by the method described by Sakamoto *et al.*[14] with minor modifications. Each membrane preparation was suspended in 10mM EGTA, 1 mg/ml bacitracin and 5 mg/ml BSA (HEPES buffer). One-half milliliter of the membrane suspension (300 mg crude membrane protein) containing 25 pM ^{125}I-CCK-8 or ^{125}I-human gastrin I (Amersham, Bucks, UK) was incubated at 24°C for 60 min with or without various concentration of unlabeled human gastrin I or CCK-8 (Peninsula, Belmont, CA). Bound and free hormones were separated by centrifugation at 10,000 x g for 5 min, and radioactivity bound to the pellets was counted. Nonspecific binding was assessed as the fraction of label that remained bound in the presence of 10^{-7}M CCK-8.

Expression of gastrin receptor cDNA in CHO-K1 cells:

A 1.8-kb BamHI fragment of the gastrin receptor cDNA from the ECL carcinoid tumor of *Mastomys* [12] was subcloned into the BamHI or SalI cloning site of the expression vector pHβAPr1neo.[5] CHO-K1 cells (ATCC, No. CCL-61, Rockville, MD), grown in Ham's F-12 medium with 10% fetal calf serum and kanamycin (100 mg/ml) were employed for transfection studies. Approximately 30,000 cells in 6-cm dishes were transfected with 6 mg of plasmid DNAs by the Chen-Okayama method.[2] G418-resistant cells were then selected in the presence of 700 mg/ml Geneticin (GIBCO, Grand Island, NY) for 4 weeks. The bindig studies with these transfected cells were performed by the same method as described above.

RT-PCR:

Five microliters of total RNA extracted from whole brain or gastric parietal cells of *Mastomys* was used to synthesize the first strand cDNA by a SUPER SCRIPT preamplification system (Bethesda Research Laboratiries, Inc., Gaithersburg, MD). The first strand cDNA was then amplified directly by the PCR method with several 17-mer primers synthesized according to the sequence of the gastrin receptor cDNA of *Mastomys* ECL carcinoid tumor.[12] The amplified cDNA fragments were sequenced by Sequenase Version 2.0 (United States Biochemical, Cleveland, OH).

Southern blot analysis:

Recently, we cloned CCK-B receptor cDNA from human brain.[8] Using not only this cDNA but also a 0.2-kb cDNA (nucleotide positions 345-519) of the *Mastomys* gastrin receptor[12] as probes, Southern blot analysis of human placental genomic DNAs was performed. Genomic DNA was extracted as described previously with minor modification.[11] Twenty micrograms of DNAs was digested with EcoRI, BamHI or HindIII, electrophorased, and capillary-transfered to nitrocellulose filters. The filters were hybridized in a buffer containing 30% w/v formamide, 5 x SSC (1 x SSC is 0.15 M NaCl/0.015 M sodium citrate, pH 7.0), 1 x Denhardt's solution, 5 mM NaH_2PO_4 / 0.1% SDS and 100 mg/ml salmon sperm DNA and the ^{32}P-laveled cDNA probes at 42°C for 18 h. The two cDNAs were ^{32}P-labeled for the probes using a nick-translation kit (1 x 10^9 cpm/mg, Amersham, Bucks, UK). Then the filters were washed with 2 x SSC twice at room temperature and 1 x SSC twice at 50°C and autoradiographed for 24 h.

RESULTS AND DISCUSSION

As we reported previously,[4] not only ^{125}I-human gastrin I (gastrin) but also ^{125}I-CCK-8 bound specifically to the *Mastmys* ECL carcinoid tumor membranes, and the specific binding of both ligands to the membrane was displaced in a concentration-dependent manner by unlabeled CCK-8 and gastrin with almost equal potencies (Fig. 1 and Table 1). Interestingly, we found in this study that both ^{125}I-CCK-8 and ^{125}I-gastrin also bound to the gastric parietal cell membrane of *Mastomys*, and like those to the carcinoid tumor membranes, both bindings were inhibited dose-dependently and equipotently by CCK-8 and gastrin (Fig. 1). On the parietal cell membrane, IC_{50} values of CCK-8 and gastrin were 140+/-26 pM and 241+/-51 pM for ^{125}I-CCK-8 binding, respectively, and 136+/-22 pM and 254+/-48 pM for ^{125}I-gastrin binding, respectively (Fig.1 and Table 1). Thus,

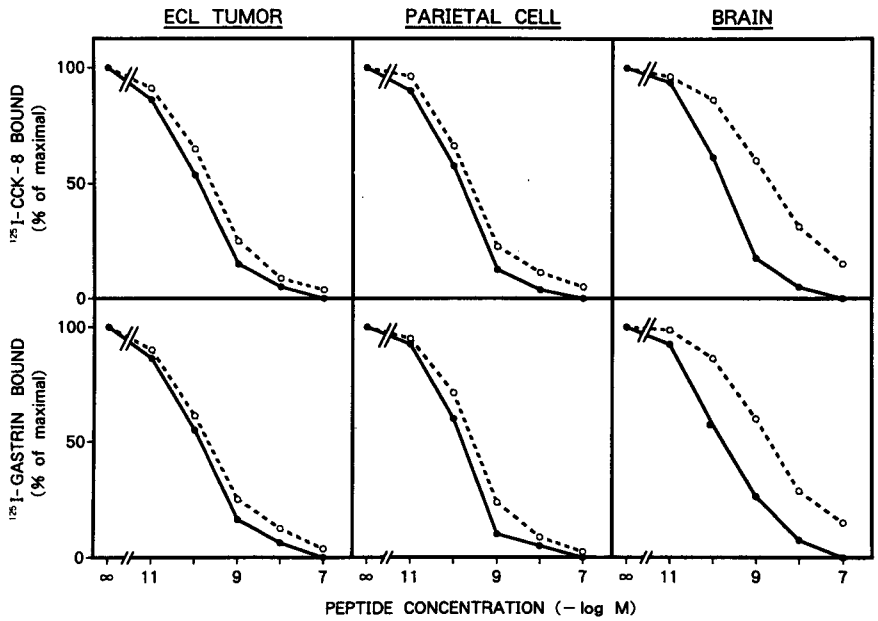

Fig.1: Effects of CCK-8 and human gastrin I on the binding of ^{125}I-CCK-8 and ^{125}I-human gastrin I to the *Mastomys* ECL carcinoid tumor, isolated parietal cell and brain membranes. Membranes were incubated with 25pM ^{125}I-CCK-8 or ^{125}I-human gastrin I in the presence or absence of increasing concentration of CCK-8 or human gastrin I for 60 min at 24°C. Specific saturable binding was expressed as the percentage of the maximal binding. Values are means of triplicate determinations from a single experiment and the data are virtually identical to those obtained in four other experiments.

CCK-8 (●——●); human gastrin I (○------○).

the binding specificity of the gastrin receptor on the parietal cell membranes for unlabeled CCK-8 and gastrin was virtually identical to that of the ECL carcinoid tumor membranes. In contrast to these two receptors, however, CCK-8 was approximately 10-20 times as potent as gastrin in inhibiting type binding of not only ^{125}I-CCK-8 but also ^{125}I-gastrin to the *Mastomys* brain membranes under the same experimental conditions (Fig.1 and Table 1), confirming the previous data obtained by using various species.[1,6,7,13,14]

Table 1: IC_{50} values of CCK-8 and human gastrin I for specific binding of ^{125}I-CCK-8 or ^{125}I-human gastrin I to *Mastomys* ECL carcinoid tumor, parietal cell and brain membranes and the transfected CHO-K1 cells.

	IC_{50} (pM)	
	CCK-8	human gastrin I
ECL carcinoid tumor		
^{125}I-CCK-8	134 +/- 22	238 +/- 46
^{125}I-gastrin	126 +/- 18	226 +/- 42
Parietal cells		
^{125}I-CCK-8	140 +/- 26	241 +/- 51
^{125}I-gastrin	136 +/- 22	254 +/- 48
Brain		
^{125}I-CCK-8	162 +/- 32	2,200 +/- 490*
^{125}I-gastrin	168 +/- 36	2,080 +/- 460*
Transfected CHO-K1 cells		
^{125}I-CCK-8	136 +/- 26	256 +/- 49
^{125}I-gastrin	138 +/- 29	238 +/- 56

IC_{50} values were defined as the concentration of unlabeled CCK-8 or human gastrin I causing half-maximal inhibition of the specific binding of ^{125}I-CCK-8 or ^{125}I-human gastrin I. Values are means +/- SE of five separate experiments. Each experiment was done in triplicate.

* Significantly different from respective value with unlabeled CCK-8 ($P < 0.01$)

Recently, we have cloned gastrin receptor cDNA from the ECL carcinoid tumor of *Mastomys*.[12] When this gastrin receptor cDNA (M-GR 10) was introduced into CHO-K1 cells, both ^{125}I-CCK-8 and ^{125}I-gastrin bound to all CHO transfectants but not to the parent CHO cells, and CCK-8 and gastrin showed almost the same potencies in displacing the specific binding of ^{125}I-CCK-8 as well as ^{125}I-gastrin (Fig.2), confirming our previous data obtained with the transfected COS cells.[12] This binding specificity of the cloned gastrin receptor for CCK-8 and gastrin is almost similar to that of the native gastrin receptors on both the ECL carcinoid tumor membrane and the parietal cell membrane of *Mastomys*, but is clearly distinct from that on the brain membrane. From these data, it can be speculated that in *Mastomys* the gastrin receptors of the ECL carcinoid tumor are identical to those of parietal cells, but are different from those of the brain.

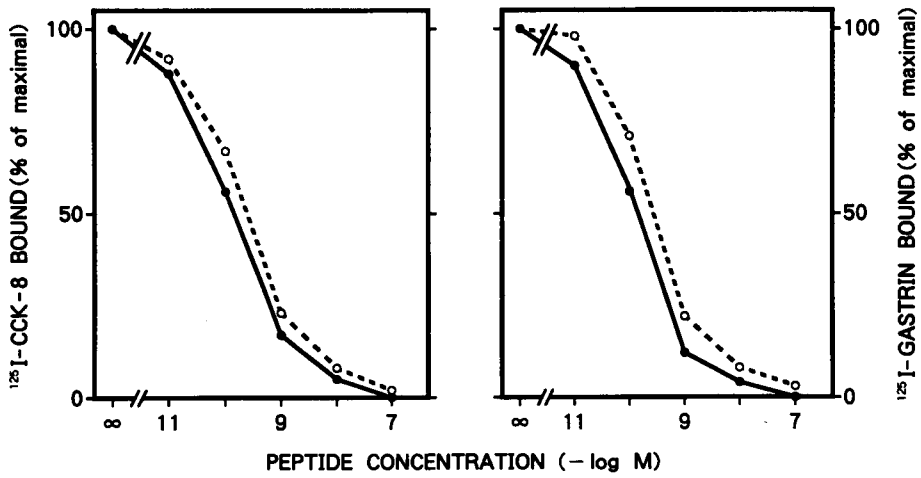

Fig.2: Inhibition of ^{125}I-CCK-8 or ^{125}I-human gastrin I binding to transfected CHO-K1 cells by CCK-8 and human gastrin I. The cells were incubated with 25pM ^{125}I-CCK-8 or ^{125}I-human gastrin I in the presence or absence of various concentrations of unlabeled peptides for 60min at 24°C. Values are means of triplicate samples from a representative of five separate experiments. • CCK-8 (●——●); human gastrin I (○------○).

In order to confirm this possibility, we tried to sequence gastrin receptor cDNAs described from both the isolated parietal cell and brain mRNAs using the RT-PCR technique. For this purpose, we synthesized several sets of oligonucleotide primers according to the sequence of the *Mastomys* gastrin receptor cDNA,[12] including those with sequences almost identical to those of rat pancreatic CCK-A receptors (one nucleotide difference).[18] By repeating the RT-PCR many times, we found that the nucleotide sequences of gastrin receptor cDNAs derived not only from the isolated parietal cells but also the brain are identical to that of the ECL carcinoid tumor, and moreover, we were unable to detect any distinct nucleotide sequence closely related to the gastrin receptor cDNA of the ECL carcinoid tumor. These results strongly suggest that all of the gastrin receptors of the ECL carcinoid tumor and the parietal cells and CCK-B receptors of the brain are products of the same gene. Indeed, Southern blot analysis of human placental genomic DNAs using the 0.2-kb cDNA of *Mastomys* gastrin receptor [12] as a probe under conditions of low stringency failed to disclose any signals other than a single discrete band hybridizing with a human brain CCK-B receptor cDNA probe corresponding to the *Mastomys* gastrin receptor probe. (Fig.3) Although obtained using human genomic DNA, these data also support the idea that CCK-B/gastrin receptors in different tissues are encoded by a single gene.

Why, then, is the binding specificity of the native brain gastrin/CCK-B receptor for CCK-8 and gastrin clearly different from that of the cloned gastrin receptor? Recently, Wank *et al.* [19] have demonstrated that in addition to gastrin/CCK-B receptor mRNA, CCK-A receptor mRNA transcripts are present in rat brain. Thus, the fact that CCK-8 is more potent than gastrin in displacing the specific binding of the two radioligands from the brain membrane could be due to the coexistence of gastrin/CCK-B receptors and CCK-A receptors in the brain. If this is the case, in addition to gastrin/CCK-B receptors, a certain amount of ^{125}I-CCK-8 should bind to CCK-A receptors, in contrast to ^{125}I-gastrin, and since the affinity of the CCK-A receptor for gastrin is very low as compared with that of the gastrin/CCK-B receptor, the IC_{50} values of gastrin for the specific bindings of ^{125}I-CCK-8 should be higher than that of ^{125}I-gastrin. Interestingly, however, the present study clearly demonstrated that the IC_{50} values of gastrin for the specific bindings of ^{125}I-CCK-8 and ^{125}I-gastrin are almost equal(Fig.1 and Tabel 1), suggesting that involvement of CCK-A receptors in our present binding experiments was negligible. In support of this idea, Wank *et al.* [19] were unable to detect any CCK-A receptor transcripts in the brain by Northern blot analysis, indicating the presence of very few CCK-A receptors in the brain. Thus, it remains to be elucidated whether the discrepancy in the binding characteristics between the native gastrin/CCK-B receptors of the brain and the gastrin receptor on the parietal cell or on the ECL carcinoid tumor of *Mastomys* results from different post-translational modification on the same receptor proteins, for example by glycosylation, or alteration of the environment surrouding the receptor, such as the lipid composition of the membrane in differnt tissues.

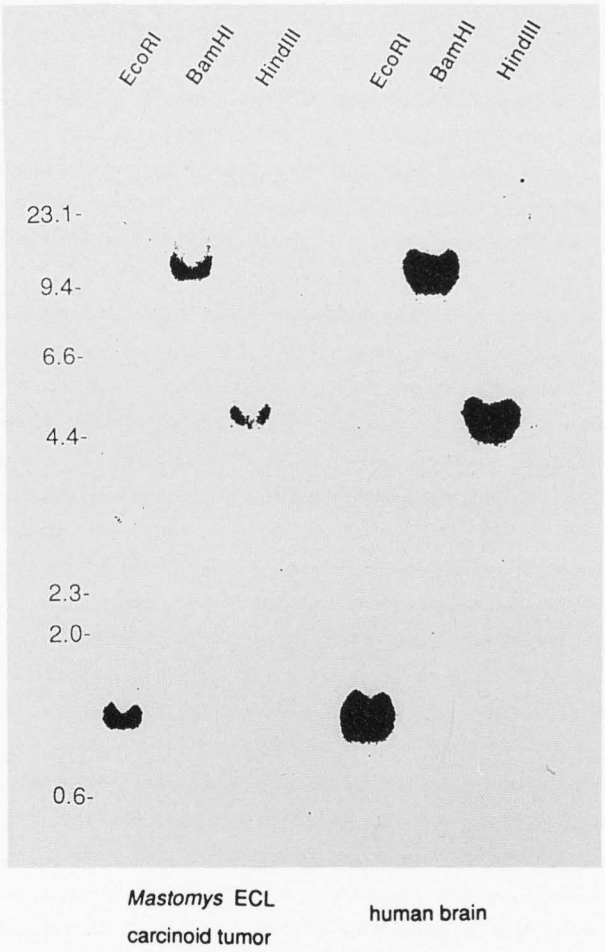

Fig.3: Southern blot analysis of human placental genomic DNAs. Genomic DNA from human placenta was digested with EcoRI, BamHI or Hind III. Both gastrin receptor cDNA from *Mastomys* ECL carcinoid tumor (left) and CCK-B receptor cDNA from human brain (right) were ^{32}P-labeled and used as probes.

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