Developmental expression and biological activity of gastrin-releasing peptide and its receptors in the kidney

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Dumesny, Chelsea, Jane C. Whitley, Graham S. Baldwin, Andrew S. Giraud, and Arthur Shulkes. Developmental expression and biological activity of gastrin-releasing peptide and its receptors in the kidney. Am J Physiol Renal Physiol 287: F578–F585, 2004.—Mammalian gastrin-releasing peptide (GRP) has a widespread distribution and multiple stimulating effects on metabolism, release of regulatory peptides, gastrointestinal and pancreatic secretions, and behavior. GRP is a potent mitogen for a number of tumor types, including colon and lung. Although GRP is known to stimulate the growth of renal tumors, little is known of its synthesis, distribution, and receptors in the developing and mature kidney. Both Northern blot analysis and RT-PCR revealed the presence of GRP mRNA in ovine kidney from midgestation through to adulthood. GRP mRNA was detected in rat kidney from embryonic day 19 to postnatal day 30 by RT-PCR. Sequence-specific radioimmunoassay demonstrated the presence of substantial amounts of fully processed amidated GRP in the ovine renal cortex and medulla. The mRNA for the major receptor subtype, GRP-R, was present in fetal and adult sheep and rat kidneys. The mRNA for the low-affinity GRP receptor, bombesin receptor subtype-3 (BRS-3), was only detected in the rat kidney. In the ovine kidney, immunohistochemistry localized GRP predominantly to the thick ascending limb of the loop of Henle. mRNAs for GRP, GRP-R, and BRS-3 were detected in the human embryonic kidney cell line HEK293, and radioimmunoassay of cell extracts and conditioned media revealed the presence of proGRP but not the amidated form. However, amidated GRP did stimulate the proliferation of these cells. These studies demonstrate that the developing and mature kidney may be previously unidentified sites of autocrine or paracrine action for GRP.

bombesin; bombesin receptor subtype-3; thick ascending limb

THE MAMMALIAN HOMOLOG of bombesin, gastrin-releasing peptide (GRP), was first isolated in the pig stomach and named for its ability to stimulate gastrin. GRP is now known to be widely distributed throughout the gastrointestinal tract and central nervous system. GRP modulates the secretion of a variety of gastrointestinal hormones and also affects pancreatic secretion, intestinal transit, muscle contractility, metabolism, and behavior (reviewed in Ref. 2). GRP plays an important role in the regulation of fetal lung development and is an autocrine growth factor in small cell lung carcinoma (7, 5). GRP was subsequently implicated as an autocrine or endocrine growth factor in a wide range of other cancers, including medullary thyroid carcinoma and carcinoma of the pancreas, stomach, breast, prostate, and colon (reviewed in Ref. 24). Two GRP receptors have been identified in mammals: GRP receptor (GRP-R) and bombesin receptor subtype-3 (BRS-3) (18). Both these receptors are members of the G protein-coupled, seven-transmembrane receptor superfamily but differ in that GRP-R has a high affinity for GRP, whereas BRS-3 has a low affinity for GRP. No naturally occurring high-affinity ligand for BRS-3 has been identified (9, 27).

There is accumulating evidence linking gastrointestinal hormones to renal function (6, 31) and renal tumorigenesis (15, 23). For instance, both gastrin and vasoactive intestinal peptide are natriuretic and have been implicated in the natriuresis associated with a meal or with sodium loading (6, 31). Receptors for gastrin and VIP are detected on both normal kidney (3, 31) and kidney tumors (26). In relation to GRP and the kidney, we have recently demonstrated the presence of large amounts of GRP mRNA and GRP immunoreactivity at a single time point in the development of fetal sheep kidney (37). The observation that the addition of GRP stimulated the proliferation of fetal kidney explants (1) is also consistent with the suggestion that GRP may play a role in the development of the kidney, as it does in lung (7). The high-affinity GRP receptor, GRP-R, has been detected in renal carcinomas although not in normal kidney (23). The finding that GRP stimulated proliferation of a renal carcinoma cell line (23) also indicates a potential role for GRP in renal carcinoma.

To further understand the relationship between GRP, kidney development, and function, we have compared the fetal and adult expression of GRP and its receptors in sheep and rat kidney. The sheep was chosen because the gestational profile is similar to man and the rat because it is frequently the model of choice for developmental studies. The cellular location of GRP was determined by immunohistochemistry, peptide expression by region-specific radioimmunoassay, and mRNA measurement and receptor expression by Northern blot analysis and RT-PCR. Finally, the biological activity of GRP was determined in an embryonic human kidney cell line.

MATERIALS AND METHODS

Cell Lines

HEK293 cells (American Type Culture Collection, Manassas, VA) were grown in monolayer cultures in Dulbecco’s modified Eagle’s medium (Invitrogen, Mulgrave, Victoria, Australia) supplemented with 10% fetal bovine serum (FBS) in an atmosphere of 95% air and 5% carbon dioxide at 37°C. Cultures were passaged at 2- to 3-day intervals to maintain the cells at subconfluent densities.

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Preparation of Total RNA

Tissue. Total RNA was prepared from 200 mg frozen tissue from adult ovine and rat whole kidney, renal medulla and renal cortex, and neonatal rat and fetal ovine kidney tissues, using Trizol reagent (GIBCO-BRL, Mulgrave, Vic., Australia). Contaminating endogenous RNases in RNA samples that were to be used in Northern analyses was inactivated by treatment with 40 μg proteinase K (Sigma, Castle Hill, NSW, Australia) in a reaction buffer consisting of 0.1 M Tris-HCl, pH 7.8, 0.05 M EDTA, and 1% SDS for 30 min at 50°C. Proteinase K was removed by extraction with an equal volume of phenol-chloroform-isomyl alcohol (25:24:1, by vol), and the RNA was precipitated with 0.3 M sodium acetate, pH 5, and 1 vol of isopropanol and resuspended in sterile water. Samples that were used for RT-PCR were treated with DNAse I to remove any genomic contamination. A 5-μg sample of each RNA sample was subjected to denaturing electrophoresis through low formaldehyde-containing agarose gel before vacuum transfer (VacuGene Pharmacia, Baulkham Hills, NSW, Australia) in 20X standard saline citrate (SSC; 300 mM NaCl, 30 mM sodium citrate) for 4 h to a nylon membrane (Hybond N++; Amersham, Baulkham Hills, NSW, Australia). After transfer, the membrane was rinsed briefly in 2X SSC and air dried before the nucleic acids were separated by agarose gel electrophoresis (as above) before vacuum transfer. Chloroform was added, and the sample was mixed and incubated at 30°C for 2 min before being spun at 12,000 g for 15 min at 4°C to separate the organic and aqueous phases. Isopropanol was added to the aqueous phase, and the sample was centrifuged at 12,000 g for 10 min at 4°C to precipitate the RNA. The RNA was washed in 75% ethanol and then resuspended in water that had been treated with diethylpyrocarbonate.

Northern Blot Analysis

Samples of total RNA (10, 20, or 30 μg as indicated) were separated by agarose gel electrophoresis (as above) before vacuum transfer (VacuGene Pharmacia, Baulkham Hills, NSW, Australia) in 20X standard saline citrate (SSC; 300 mM NaCl, 30 mM sodium citrate) for 4 h to a nylon membrane (Hybond N++; Amersham, Baulkham Hills, NSW, Australia). After transfer, the membrane was rinsed briefly in 2X SSC and air dried before the nucleic acids were fixed to the membrane by treatment with ultraviolet light (700 nm) (UV Cross-Linker; Amersham). Labeled riboprobes for GRP and GAPDH were prepared and purified essentially as described previously (38) using the MAXIscript kit (Genset, Adelaide, SA, Australia) including 0.85 μM [32P]UTP and 2.15 μM unlabeled UTP. Recombinant plasmids containing ovine GRP cDNA or ovine GAPDH cDNA (exons A–H; a generous gift from Dr. Louise Baker, Dept. of Veterinary Science, Univ. of Melbourne) were digested with appropriate restriction enzymes to yield labeled antisense cRNA molecules of ~230 nt and 290 nt, respectively. Riboprobes were added [5 × 10^5 counts·min⁻¹ (cpm)·μl⁻¹] to membranes sandwiched between two sheets of Whatman no. 1 filter paper (Medos, Mt. Waverly, Vic., Australia) that had been prehybridized for 16 h at 68°C in RNA-RNA hybridization buffer (55% formamide, 5X Denhardt’s solution, 2X SSC, 50 mM sodium phosphate, pH 6.8, 100 μg/ml denatured salmon sperm DNA, 100 μg/ml Escherichia coli tRNA, and 1% SDS). After hybridization at 68°C for 24 h, membranes were briefly rinsed twice in 0.1X SSC-0.1% SDS and washed for 15 min at 68°C in the same solution. GRP or GAPDH mRNA was detected after autoradiography at ~70°C using Kodak XAR-5 film (Amersham Biosciences) and two intensifying screens.

Table 1. Primers used for detection of GRP, GRP-R, and BRS-3 mRNA

<table>
<thead>
<tr>
<th>Primers</th>
<th>Probe</th>
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<tbody>
<tr>
<td>Rat GRP</td>
<td>5’ GGCTCCTGAAACGAGAAAAGTGG 3’</td>
</tr>
<tr>
<td>Ovine GRP</td>
<td>5’ GGTGAGGACCTCGGAGGAGA 3’ (rc)</td>
</tr>
<tr>
<td>Human GRP</td>
<td>5’ ACGCGGCTGAAACAGATGTT 3’ (rc)</td>
</tr>
<tr>
<td>Rat GRP-R</td>
<td>5’ GATGGAGATGACACATAGGACG 3’</td>
</tr>
<tr>
<td>Ovine GRP-R</td>
<td>5’ GGGAGGCGGGGACGACAACTAAT 3’ (rc)</td>
</tr>
<tr>
<td>Human GRP-R</td>
<td>5’ CGAGCTTGAGGACGAGTTTGGGACA 3’ (rc)</td>
</tr>
<tr>
<td>Rat BRS-3</td>
<td>5’ CCGGATCAATTAGGACGACGGG 3’ (rc)</td>
</tr>
<tr>
<td>Ovine BRS-3</td>
<td>5’ TGCGGATCAATTAGGACGACGGG 3’ (rc)</td>
</tr>
<tr>
<td>Human BRS-3</td>
<td>5’ CTTGAGGATCAATTAGGACGACGGG 3’ (rc)</td>
</tr>
</tbody>
</table>

M. mixed primer set containing either A or C; Y, mixed primer set containing either C or T. GRP, gastrin-releasing peptide; GRP-R, GRP receptor; BRS-3, bombesin receptor subtype-3; rc, reverse complemented.
bromide. The nucleic acids were transferred to nylon membrane (Hybond N+, Amersham Pharmacia Biotech) by capillary action in 0.4 M sodium hydroxide. Oligonucleotides (see Table 1) were labeled with $^{32}$P using terminal transferase according to the manufacturer’s instructions (Roche, Castle Hill, NSW, Australia) and used for Southern hybridization of the blot in rapid-hyb buffer (Amersham) at 42°C for 1–2 h. Unhybridized probe was removed by rinsing blots twice in 0.5× SSC-0.1% SDS at room temperature and then washing for 15 min (twice) in the same solution at 42°C. The products were visualized after exposure to X-ray film (Kodak Biomax MR; Amersham) for 2–12 h.

**Proliferation Studies**

Cells were grown in media supplemented with 10% FBS, seeded in 96-well plates at a density of 25,000 cells/well, and grown for 24 h. The cells were then serum starved for 24 h before being treated with different concentrations of bombesin in media supplemented with 1% FBS for 3 days. Fifteen microliters of 5 ng/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma Aldrich, Castle Cove, NSW, Australia) was prepared using bombesin (2,000 fmol/ml) with an ID$_{50}$ (50% inhibition of binding) of 100 fmol/tube. The intra- and interassay coefficients of variation were 4% and 14%, respectively.

**NH$_2$-terminal assay.** Antiserum N798 was raised against the NH$_2$-terminal 11-amino acid sequence of human proGRP [equivalent to GRP(1–11)] conjugated to keyhole limpet hemocyanin. As expected, this antiserum (used at a final concentration of 1:2,000) detects human GRP(1–11) and GRP(1–27) but not GRP(18–27). Because of sequence differences at the NH$_2$ terminus, antiserum N798 does not detect rat or sheep GRP. GRP(1–11) (Auspep, Melbourne, Vic., Australia) was used for the standard curve, and the tracer was $^{125}$I-labeled GRP(1–11), which was prepared using a modified iodogen method and purified by reverse-phase HPLC. The ID$_{50}$ was 200 fmol/tube.

**Immunohistochemistry**

Cross-sections (4 μM) of ovine kidney, fixed for 16–24 h in fresh Bouin’s fixative or 4% paraformaldehyde and embedded in paraffin, were stained using the anti-bombesin primary antiserum R40 and biotinylated anti-rabbit secondary antibody (DAKO, Botany, NSW, Australia) and visualized using avidin horseradish peroxidase (Vector, Burlingame, CA) and 3,3'-diaminobenzidine (Sigma, St. Louis, MO) with hematoxylin counterstaining. Antiserum R40 was raised in a rabbit against synthetic amphibian bombesin and used at a dilution of 1:2,000–1:4,000. Controls included omission of the primary antibody and absorption of the primary antibody overnight with either ovine GRP(1–27) (50 μM) or Lys$^2$-bombesin (500 μM) before staining.

**Statistics**

The data were analyzed by one-way ANOVA followed by Dunnett’s or Bonferroni tests for multiple comparisons.

**RESULTS**

**GRP mRNA in Fetal and Adult Ovine Kidney**

The ontogeny of GRP mRNA expression in whole ovine fetal kidney was examined by Northern analysis in tissue at 60, 80, 100, 120, 135, 140, and 141 days of gestation (term is 145 days) (Fig. 1A). Expression was low but detectable at days 60, 80, and 100 and increased substantially at day 135, reaching a maximum in the adult. Expression of GRP mRNA in the renal medulla of the adult sheep was higher than in the cortex (Fig. 1B). RT-PCR confirmed the presence of GRP mRNA in the fetal kidney from as early as 45 days of gestation (Fig. 1C).

**Detection of GRP mRNA in Fetal and Adult Rat Kidney**

Because of the relatively small amounts of rat tissue, RT-PCR of total RNA was used for detection of GRP mRNA. GRP mRNA was detected in rat kidney from embryonic day 19 to postnatal day 50 (Fig. 2). However, in contrast to the sheep, no GRP mRNA was found in the adult rat kidney, although GRP mRNA was found in the adult gastric fundus (data not shown). GAPDH or β-actin PCR product was observed for all cDNA samples tested, except genomic and water controls (data not shown).

**Detection of GRP-R and BRS-3 Receptor mRNA in Fetal and Adult Sheep Kidney and in Rat Kidney**

A single GRP-R PCR product of the expected size was observed by ethidium bromide staining when cDNA prepared from either ovine or rat fetal kidney tissues or adult tissues was used as template. The identity of the product was confirmed by Southern hybridization using an internal oligonucleotide probe.
Expression was detected at all time points in rat (Fig. 3A) and sheep (Fig. 3C) fetal kidneys. In the adult kidney, both the cortex and medulla of rat and sheep expressed GRP-R (Fig. 3B and D). BRS-3 mRNA was also detected by RT-PCR in rat fetal kidney tissues (Fig. 4A). In the adult rat kidney, both the cortex and medulla expressed BRS-3 (Fig. 4B). No BRS-3 PCR product could be detected in any ovine fetal or adult kidney, although BRS-3 expression was detected in ovine pituitary (data not shown).

**GRP Immunoreactivity in Ovine Kidney**

Extracts of ovine cortex and medulla contained significant amounts of GRP immunoreactivity when measured with the antiserum R40 (Fig. 5). This antiserum is directed to the amidated COOH terminus of GRP(1–27). The concentrations were one-half to one-third of those found in the stomach, the major known source of GRP.

**Cellular Distribution of GRP Immunoreactivity in the Sheep Kidney**

GRP staining was more pronounced in Bouin’s fixed tissues than those fixed in 4% paraformaldehyde, even when antigen

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**Fig. 1.** Expression of gastrin-releasing peptide (GRP) mRNA in ovine kidney. Total RNA (10 μg) from ovine fetal kidney [A; 60, 80, 100, 120, 135, 140, and 141 days (D) and newborn and adult, as indicated] and ovine renal medulla and cortex (B) was fractionated by denaturing gel electrophoresis, transferred to a nylon membrane, and hybridized with radiolabeled recombinant plasmids encoding ovine GRP (top panels) or GAPDH (bottom panels). C: template cDNA was prepared from RNA isolated from ovine fetal kidney [40–45, 80, 100, 135, 140, and 141 days (d) and newborn and adult]. The cDNA was subjected to PCR (30 cycles), samples were electrophoresed on an agarose gel, and the products were visualized by Southern hybridization using a radiolabeled GRP-specific oligonucleotide probe.

**Fig. 2.** Expression of GRP mRNA in rat kidney tissue. Template cDNA was prepared from RNA isolated from the developing rat kidney [embryonic (E) day 19; postnatal (PN) days 1, 5, 10, 20, and 30] and adult rat kidney. The cDNA was subjected to PCR (30 cycles), samples were electrophoresed on an agarose gel, and the products were visualized by Southern hybridization using a radiolabeled GRP-specific oligonucleotide probe.

**Fig. 3.** Detection of GRP receptor (GRP-R) in ovine and rat kidney tissue. Template cDNA was prepared from RNA isolated from the developing rat kidney (A; embryonic day 19; postnatal days 1, 5, 10, 20, and 30 and adult), adult rat kidney (B), ovine fetal kidney (C; 60, 80, 100, 120, 135, 140, and 141 days and newborn and adult, as indicated), and ovine adult kidney (D) and was subjected to PCR (30 cycles, rat; 40 cycles, ovine) for the detection of GRP-R. PCR samples were electrophoresed on agarose gels, and the products were visualized by Southern hybridization using a radiolabeled GRP-R-specific oligonucleotide probe.

**Fig. 4.** Detection of bombesin receptor subtype-3 (BRS-3) in rat kidney tissue. Template cDNA was prepared from RNA isolated from the developing rat kidney (A; embryonic day 19; postnatal days 1, 5, 10, 20, and 30) and adult rat kidney (B) and was subjected to PCR (30 cycles, developing kidney; 35 cycles, adult kidney) for the detection of BRS-3. PCR samples were electrophoresed on agarose gels, and the products were visualized by Southern hybridization using a radiolabeled BRS-3-specific oligonucleotide probe.
retrieval was applied to the latter; however, the same structures were stained using either fixative. GRP immunoreactivity was ablated when the primary antibody was preincubated with ovine GRP(1–27) (50 μM) or Lys3-bombesin (500 μM) (data not shown) or when the primary antibody was omitted (Fig. 6A).

GRP-positive epithelial cells were observed in both the renal cortex and the medulla. The glomerular capsule and renal corpuscle were unstained; however, cells lining the thick ascending limb of the loop of Henle were strongly immunoreactive (Fig. 6, B and C). The proximal tubules and distal tubules were more weakly stained. In the medulla, the cells staining most strongly were those lining the thick ascending limb, situated in the inner stripe of the outer medulla, with weaker staining of the collecting duct (Fig. 6, D and E). A striking finding was the clear and abrupt demarcation in GRP immunoreactivity between the ascending thin limb of the inner medulla (GRP negative) and the thick ascending limb of the outer medulla (GRP positive; Fig. 6E). Other GRP-negative structures were the cells of the renal vasculature, including the vasa recta bundles and capillaries. The staining profile was similar in a 130-day fetus, with strongest staining in the thick ascending loop of Henle (Fig. 6F).

Detection of GRP, GRP-R, and BRS-3 mRNA and GRP Immunoreactivity in HEK293 Cells

mRNA for GRP, GRP-R, and BRS-3 was detected in RNA extracted from the human embryonic kidney cell line HEK293 by RT-PCR (Fig. 7). Single ethidium bromide bands were...
observed for each PCR product. The presence of GRP immunoreactivity in HEK293 cell extracts and conditioned media was investigated by RIA with antiserum R40, which is directed to the amidated COOH terminus of GRP(1–27), and with antiserum N798, which is directed to the NH2 terminus of GRP. No GRP immunoreactivity was detected with the COOH terminal assay. However, antiserum N798 detected significant immunoreactivity in cell extracts (6.0 ± 0.6 fmol/10^6 cells), indicating the presence of nonamidated forms of GRP.

**Proliferation Studies**

The colorimetric MTT assay was used to determine whether bombesin stimulated mitogenesis in HEK293 cells. Bombesin at concentrations of 1 nM and 10 nM significantly stimulated proliferation by 16–25% above the DMSO control (P < 0.05; Fig. 8).

**DISCUSSION**

Recently, we reported the presence of GRP mRNA in a wide variety of ovine fetal tissues (fundus, colon, jejunum, ileum, duodenum, kidney, adrenal, lung, heart, and pancreas) with particularly high concentrations measured in the kidney at a single time point (37). Because of the unexpected finding of GRP mRNA in the fetal kidney, we have in the present study determined the expression of GRP mRNA and peptide, and of the two GRP receptors, during ontogeny. We report the presence in sheep and rat kidney of both GRP mRNA and peptide and the expression of the two major GRP receptor subtypes. Thus GRP has the potential to be an autocrine regulatory factor in the kidney. This role may include regulation of growth because we further showed that GRP stimulated proliferation of a human embryonic kidney cell line.

The GRP regulatory system (peptide plus receptors) was present in fetal and neonatal kidney of both rat and sheep. Previous studies have shown GRP plays a role in normal fetal lung growth and maturation in a number of species, including humans, baboons, rats, and mice (7, 17). Peak GRP mRNA expression occurs during the canalicular phase of pulmonary development in the lung, which is approximately midgestation (28). In ovine fetal kidney, substantial expression was seen by day 135 (late gestation). The rat kidney contained GRP mRNA between embryonic day 19 and postnatal day 30, but GRP mRNA was absent from the adult kidney. Interestingly, the adult rat gastric fundus contained GRP mRNA, suggesting an organ-specific developmental profile and an endocrine mode of action in the adult, with a renal paracrine or autocrine effect in the fetus. Given the presence of GRP and its receptors in fetal kidney and its known role in lung development, it is plausible that GRP may also have a role in kidney development.

There is little information on GRP expression in the normal adult kidney. A study of the literature reveals several associations between GRP and renal function and dysfunction but no evidence that GRP is produced locally. Several studies have shown increased plasma GRP or proGRP in patients with chronic renal failure (12, 13, 16), but this is probably the result of impaired metabolism and excretion. Our data provide the first evidence for the presence of GRP mRNA in normal adult kidney. Furthermore, the mRNA was being translated into peptide as demonstrated by a specific RIA. In contrast to the sheep, no GRP mRNA was detected in the adult rat kidney although it was abundant in the fetal rat kidney.

RIA of normal ovine kidney extracts revealed GRP immunoreactivity in both the cortex and medulla, with slightly higher immunoreactivity in the cortex. Immunohistochemistry showed that the majority of staining in both the fetal and adult sheep was in cells of the thick ascending limb of the loop of Henle, which is involved in the active transport of NaCl from the lumen to the surrounding interstitium. The reabsorption is mediated by a Na-K-2Cl cotransporter with energy provided by the Na^+–K^+–ATPase located in the basolateral membrane (8). The activity of this enzyme and the extent of sodium reabsorption can be modified by a large number of hormones and mediators such as vasopressin, glucagon, calcitonin, and adrenergic agonists (8). In terms of gastrointestinal peptides, both gastrin (31) and VIP (6) are natriuretic, although they are not synthesized by the kidney, and corresponding changes in renal Na^+–K^+–ATPase have not been reported. There are no reports on the effect of GRP on renal electrolyte transport, although it

**Fig. 7.** Expression of mRNA for GRP, GRP-R, and BRS-3 in HEK293 cells. Template cDNA was prepared from RNA isolated from HEK293 cells and subjected to PCR (30 cycles, GRP and BRS-3; 40 cycles, GRP-R) for detection of GRP (A), GRP-R (B), and BRS-3 (C) mRNA. PCR samples were electrophoresed on agarose gel, and products were visualized by Southern hybridization using radiolabeled gene-specific oligonucleotide probes.

**Fig. 8.** Effect of bombesin (Bn) on cell proliferation in HEK293 cells. Proliferation was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay, and results (means ± SE of 5 different experiments, performed in sextuplicate) are expressed as percent control. *P < 0.05 compared with control.
is a net stimulant of sodium secretion in the colon (30). The demonstration in the present study that GRP and its receptor are present in the kidney as well as the intestine supports the concept of a local paracrine/autocrine effect on renal function as well as a potential enteric-renal link. A relevant precedent is that of peptides of the guanylin/uroguanylin family, which are synthesized in both intestine and renal tubules and have profound effects on renal electrolyte homeostasis (20).

Evidence for the expression of receptors for GRP in normal kidney is conflicting. One study identified GRP-R mRNA and peptide in human renal carcinomas and derived cell lines but not in normal human kidney (23). However, GRP-R mRNA has been found in a monkey kidney cell line (14), and in the present study we detected GRP-R mRNA in adult and fetal sheep kidney and in rat kidney. This is consistent with a study that showed GRP-R gene expression from E-16 to E-20 in rat embryonic kidneys (32). It has also been shown that GRP-R mRNA is expressed in early embryonic stages in various other organs, including the brain, the urogenital, respiratory, and digestive tracts (32), and the developing lung (33). GRP-R knockout mice have increased food intake and are heavier, but the role of this receptor in renal function has not been determined (19).

BR-S-3 mRNA was found in rat adult and fetal kidney tissues but not in sheep. This is the first report of the presence of this receptor in the kidney. The absence from ovine kidney may seem surprising, as there is abundant expression of BR-S-3 in ovine hypothalamus and pituitary (36). However, previous studies have shown that the expression of BR-S-3 mRNA is highly species and organ specific. For instance, BR-S-3 mRNA expression is found in pregnant uterus of guinea pig (10) and human (11, 35) but not in mouse (21) or sheep uterus (36). BR-S-3 knockout mice have a similar although more pronounced phenotype than GRP-R knockout mice, as they are hyperphagic and obese (39). The effect on renal function was not determined in these mice.

Because of the growth factor-like properties of GRP and the presence of GRP and its receptors in ovine and rat fetal kidney tissues, we sought to determine whether GRP had any functional effects on the human embryonic kidney cell line (HEK293). We showed that these cells express mRNA for GRP, GRP-R, and BR-S-3, while only the nonamidated form of GRP peptide is present. However, nonamidated forms of GRP/bombesin may be a growth factor for the developing kidney, potentially via an autocrine regulatory loop. Similar results have been reported for gastrin in the autocrine stimulation of HEK293 growth (29).

In summary, we have demonstrated the presence of GRP and its receptors in rat and sheep kidney during development and in the adult. As with cell lines from other organs, GRP has a proliferative effect in a renal cell line. GRP may therefore be an autocrine/paracrine growth or differentiation factor in the kidney, and GRP antagonists may have a role in controlling GRP-responsive renal cell carcinoma. On the basis of the restricted distribution of GRP in the thick ascending limb of the loop of Henle, the effects of GRP on water and electrolyte transport warrant further investigation.

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GRANTS

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