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Cloning of cDNAs encoding amphibian bombesin: Evidence for the relationship between bombesin and gastrin-releasing peptide

(*Bombina bombina*/mass spectrometry/HPLC/polymerase chain reaction/prohormone processing)

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ABSTRACT **Bombesin is a tetradecapeptide originally isolated from frog skin; its mammalian homologue is the 27-amino acid peptide gastrin-releasing peptide (GRP). cDNAs encoding GRP have been cloned from diverse species, but little is yet known about the amphibian bombesin precursor. Mass spectrometry of HPLC-separated skin exudate from *Bombina orientalis* was performed to demonstrate the existence of authentic bombesin in the skin of this frog. A cDNA library was prepared from the skin of *B. orientalis* and mixed oligonucleotide probes were used to isolate cDNAs encoding amphibian bombesin. Sequence analysis revealed that bombesin is encoded in a 119-amino acid prohormone. The carboxyl terminus of bombesin is flanked by two basic amino acids; the amino terminus is not flanked by basic amino acids but is flanked by a chymotryptic-like cleavage site. Northern blot analysis demonstrated similarly sized bombesin mRNAs in frog skin, brain, and stomach. Polymerase chain reaction was used to show that the skin and gut bombesin mRNAs encoded the identical prohormones. Prohormone processing, however, differed between skin and gut. Chromatography showed the presence of only authentic bombesin in skin whereas gut extracts contained two peaks of bombesin immunoreactivity, one consistent in size with bombesin and one closer in size to mammalian GRP. Thus the same bombesin prohormone is processed solely to bombesin in skin but is processed to a peptide similar in size to bombesin and to a peptide similar in size to mammalian GRP in stomach.**

Bombesin is a 14-amino acid peptide originally isolated from the skin of the frog *Bombina bombina* (1). After its initial isolation from frog skin, bombesin was found to have multiple effects in mammals, including the induction of hypothermia, stimulation of growth and DNA replication, and stimulation of the release of many gastrointestinal hormones (2, 3). Immunohistochemistry demonstrated a wide distribution of bombesin-like immunoreactivity (BLI) in mammalian brain, gastrointestinal tract, and lung (4–6). Chromatography, however, showed that mammalian bombesin immunoreactivity consisted of two forms, one larger than amphibian bombesin and one slightly smaller than bombesin (4, 7). By using gastrin release as a bioassay, McDonald *et al.* (8) isolated a 27-amino acid peptide with strong homology in its carboxyl terminus to bombesin. This peptide isolated from porcine stomach was named gastrin-releasing peptide (GRP) and reproduced all the bioactivities of bombesin (9). Subsequently, GRP has been isolated or cloned from rat, human, canine, guinea pig, and avian species (10–14). From canine intestine, Reeve *et al.* (12) isolated the smaller form of GRP immunoreactivity and determined it to be the carboxyl-terminal decapeptide of GRP. This peptide, designated GRP-10 or GRP-(18–27), has also been isolated from human stomach and porcine spinal cord (15, 16).

In amphibians, three subfamilies of bombesin-like peptides have been described, the bombesins, the ranatensins, and the phyllolitorins (3). Each subfamily is characterized by a common amino acid near its carboxyl terminus. The bombesins have a leucine as the penultimate residue, the ranatensins have a phenylalanine as the penultimate residue, and the phyllolitorins have a serine as the third residue from the carboxyl terminus. The nonapeptide neuromedin B is the mammalian homolog of ranatensin (17); no mammalian homologs of the phyllolitorins have yet been isolated.

Frogs express many mammalian neuropeptides in their skin at exceedingly high levels. *Xenopus laevis*, for example, expresses thyrotropin-releasing hormone at levels 1000-fold higher than found in mammalian hypothalamus (18). In frog skin, peptides are localized in cutaneous epithelial glands and are secreted in response to noradrenergic stimulation (19). In frog central nervous system, neuropeptides occur at levels more consistent with their concentration in mammalian central nervous system. Thus neuropeptides appear to serve two roles in frogs; a neuroendocrine role analogous to their role in mammals and an external secretory role from skin. Consistent with this, Walsh *et al.* (20) found BLI in frog stomach and in frog brain in species that lacked bombesin in their skin. Although all frogs examined to date express BLI in stomach and brain, only *Bombina* species appear to express bombesin in skin.

In this paper we report the cloning of the cDNA for amphibian bombesin and demonstrate that the identical mRNA is expressed in skin, brain, and stomach but that, in skin, it is processed only to bombesin, whereas in stomach it is also processed to a large form, similar in size to mammalian GRP.

MATERIALS AND METHODS

Tissues. *Bombina orientalis* were obtained from California Zoological Supply (Santa Ana, CA) and maintained on crickets. For chromatography of skin and gut, tissues were extracted in trifluoroacetic acid (F_3CCOOH). For HPLC separation and mass spectrometry analysis, the frogs were injected with 0.5 ml of 1 mM norepinephrine and the induced skin secretions were collected (21).

HPLC/Mass Spectrometric Analysis. Frog skin peptides were analyzed as described (22–24). In brief, skin secretions from frogs injected with 0.5 ml of 1 mM norepinephrine were collected in 0.9% saline, lyophilized, and desalted on Sephadex G-10. Fractions between the void volume and the point

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Abbreviations: GRP, gastrin-releasing peptide; GRP-10, carboxyl-terminal decapeptide of GRP; F_3CCOOH, trifluoroacetic acid; LSIMS, liquid secondary ion mass spectrometry; PCR, polymerase chain reaction; BLI, bombesin-like immunoreactivity.

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of salt elution were pooled, lyophilized, and resuspended in 0.1% F_3CCOOH. HPLC separation was carried out on a Vydac C_{18} analytical column (25 × 0.46 cm) with a linear gradient over 100 min from 0.1% F_3CCOOH to 70% (vol/vol) acetonitrile/30% (vol/vol) H_2O/0.08% F_3CCOOH. Eluting components were monitored at 215 nm. The precise molecular weights (and partial sequences of peptides isolated by HPLC as single peaks) were determined by liquid secondary ion mass spectrometry (LSIMS) by using a Kratos model MS50S mass spectrometer with a cesium ion source (25). Eluted peptide peaks were dried under vacuum and then dissolved in H_2O containing 0.1% F_3CCOOH. Approximately 1 μl of glycerol/thioglycerol, 1:1 (vol/vol), was applied to the stainless steel probe tip and a small amount of the sample solution was added along with ≈0.1 μl of 1% F_3CCOOH. A Cs^+ ion primary beam energy of 6–8 keV was used and the secondary sample ions were accelerated at 8 kV. Scans were taken in the positive ion mode at 300 sec per decade and recorded with a Gould electrostatic recorder. Manual calibration was performed using Ultramark model 1621 as an external reference to an accuracy of ±0.2 dalton. Masses are reported for the isotopically pure component of each molecular ion cluster.

cDNA Library Construction, Screening, and DNA Sequence Analysis. *B. orientalis* were sacrificed 4 weeks after a single injection of 0.5 ml of 1 mM norepinephrine, a treatment that induces depletion of skin neuropeptides followed by a marked induction of peptide-encoding mRNAs as part of the repletion process (21). Total RNA and poly(A)$^+$ RNA were prepared from 12 dorsal skins with guanidine thiocyanate and oligo(dT) by standard methods (26). Double-stranded cDNA was prepared by the method of Gubler and Ross (see ref. 26), treated wtih *Eco*RI methylase, ligated wtih *Eco*RI linkers into the vector λZap (Stratagene), and packaged into phage. The library was amplified once and screened with mixed oligonucleotide probes complementary to the sequence of amphibian bombesin, as described in the *Results*. Hybridizing phage were plaque-purified and then excised *in vivo* into the plasmid Bluescript by following Stratagene's protocol. DNA sequence was obtained from double-stranded template (27) initially by using flanking vector sequence as primers and then by using internal sequences as primers. All sequence was obtained from at least two plasmids on both strands.

RNA Blot Analysis. For Northern blot analysis, total RNA was prepared from tissue extracts as described above. RNA was electrophoresed on formaldehyde/agarose gels (1.75%), transferred to a nylon membrane, and UV-crosslinked (26). ^{32}P-labeled antisense cRNA was transcribed from the bombesin cDNA by using T7 polymerase as described (28). Hybridization conditions were 50% (vol/vol) formamide/5× SSC/5× Denhardt's solution/50 mM sodium phosphate, pH 7.0/2.5% SDS/sonicated denatured salmon sperm (200 μg/ml)/phenol-extracted yeast RNA (200 μg/ml) at 65°C for 18 hr (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0; 1× Denhardt's solution = 0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin). Blots were washed for two 15-min periods in 2× SSC/0.2% SDS at 25°C, followed by two 30-min washes in 0.1× SSC/0.5% SDS at 65°C, and then exposed to film at −70°C with an intensifying screen.

PCR Analysis. Poly(A)$^+$ RNA from stomach and brain was reverse transcribed using oligo(dT) as a primer (28) and then amplified using two primers from the sequence of the bombesin cDNA, as shown in Fig. 2*A*. Reaction conditions were 10 mM Tris·HCl, pH 8.3/50 mM KCl/1.5 mM $MgCl_2$/0.01% gelatin/0.01% Tween 20/0.01% Nonidet P-40/each primer at 1 μM/all four dNTPs (each at 400 μM)/2.5 units of *Thermus aquaticus* polymerase (Cetus)/2–10 μl of DNA/H_2O to 100 μl (29). Temperatures were 35 cycles of 92°C for 1 min, 55°C for 2 min, and 72°C for 1.5 min, followed by holding at 4°C. The PCR products were subcloned into the vector Bluescript II (Stratagene) and sequenced as above.

Chromatography and Radioimmunoassay (RIA) of Skin and Stomach Extracts. After removal, dorsal skin and stomach were boiled for 10 min in 5 vol of H_2O, cooled on ice, homogenized, and mixed with an equal volume of 4% F_3CCOOH. After 15 min, the extract was centrifuged at

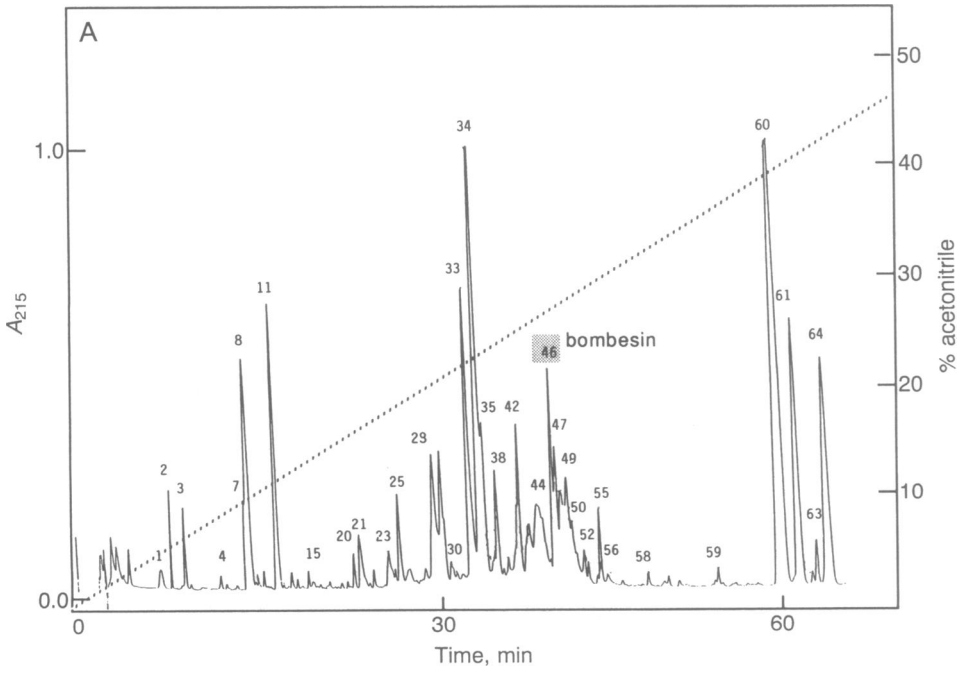

FIG. 1. HPLC and LSIMS analysis of peptides secreted from the skin of *B. orientalis*. (*A*) Secretions from frog skin were prepared with a Vydac C_{18} analytical column (25 × 0.46 cm) with a 0–70% acetonitrile gradient in 0.1% trifluoroacetic acid over 100 min with a flow rate of 1.0 ml/min. Eluting components were monitored at 215 nm (1.0 arbitrary units, full scale). (*B*) Eluting peaks were isolated and subjected to LSIMS analysis. The observed $(M + H)^+$ ions for the components of each major peak are as shown. Based on its sequence, the predicted $(M + H)^+$ for the isotopically pure component of the bombesin molecular ion is 1619.9, corresponding exactly to fraction 46.

10,000 × *g*. A portion of the supernatant (10 ml) was loaded onto a SepPak C_{18} cartridge (Waters) previously wetted with 15 ml of acetonitrile and equilibrated in 20 ml of 0.1% F_3CCOOH. After loading the column was rinsed with 5 ml of 0.1% F_3CCOOH and material was eluted with 2 ml of 50% acetonitrile containing 0.1% F_3CCOOH. One milliliter (corresponding to 0.5 g of tissue) of the eluate was mixed with 1 ml of 3% (vol/vol) acetic acid and the mixture was loaded onto a Sephadex G-50 superfine column (1 × 95 cm) equilibrated in 3% acetic acid. Fractions (1 ml) were collected and assayed for BLI as described (12).

RESULTS

The presence of authentic bombesin in the dorsal skin of *B. orientalis* was determined by HPLC and mass spectrometry. The HPLC chromatogram of purified *B. orientalis* skin secretions is shown in Fig. 1*A*. The complexity of the chromatogram shows the multiple components present in skin secretions. LSIMS analysis of individual peaks yielded protonated molecular ions $(M + H)^+$ for each fraction, as shown in Fig. 1*B*. The $(M + H)^+$ of fraction 46 was exactly equal to that predicted for bombesin (m/z = 1619.9). Sequence ions consisting of overlapping carboxyl-terminal and amino-terminal fragments of bombesin (24, 30) provided a complete confirmation of the peptide as authentic bombesin. For example, masses of m/z = 1488, 1375, 1238, 1181, 1082, 1011, 825, 697, and 583 corresponding to the bombesin carboxyl-terminal fragments of bombesin-(2–14), bombesin-(3–14), . . ., bombesin-(10–14), respectively, were identified.

To isolate cDNAs encoding amphibian bombesin, a cDNA library was prepared from the dorsal skin of *B. bombina*. Approximately 250,000 phage were screened with a 14-base 32-fold mixed oligonucleotide CCNACNGCCCAYTG, where N is any base and Y = a pyrimidine. This sequence was complementary to the RNA encoding amino acids 7–10 plus the first two bases of the codon for Gly-11 of bombesin. Hybridization conditions were 6× SSC/5× Denhardt's solution/0.1% SDS/sonicated denatured salmon sperm (200 μg/ml) at 33°C for 36 hr. Washing conditions were 6× SSC/0.1% SDS at 33°C. One hybridizing cDNA was isolated, the library was rescreened, and six more hybridizing phase were isolated. Sequence analysis revealed the structure of the bombesin prohormone, as shown in Fig. 2*A*. Bombesin is encoded in a 119-amino acid prohormone in a 684-nucleotide cDNA. The amino-terminal pyroglutamate of bombesin is made from glutamine, and the carboxyl-terminal amide is donated by glycine. A set of dibasic amino acids is at the cleavage site of the carboxyl-terminus of bombesin. The amino terminus of bombesin is not flanked by basic amino acids but is flanked by a chymotryptic-like cleavage signal, highly homologous to a sequence at the carboxyl terminus of the B chain of rat relaxin (33). The bombesin precursor contains a second dibasic cleavage site between positions 76 and 77, allowing potential production of other biologically active peptides. Data base analysis of the bombesin cDNA showed highest homology on both the nucleotide level and the amino acid level to the precursor for amphibian ranatensin (17). The homology with the ranatensin precursor is shown in Fig. 2*B*.

To determine the relation between the bombesin-like peptide expressed in skin and gut, Northern blot analysis and chromatography of BLI were performed. Northern blot analysis showed identically sized mRNAs expressed in skin,

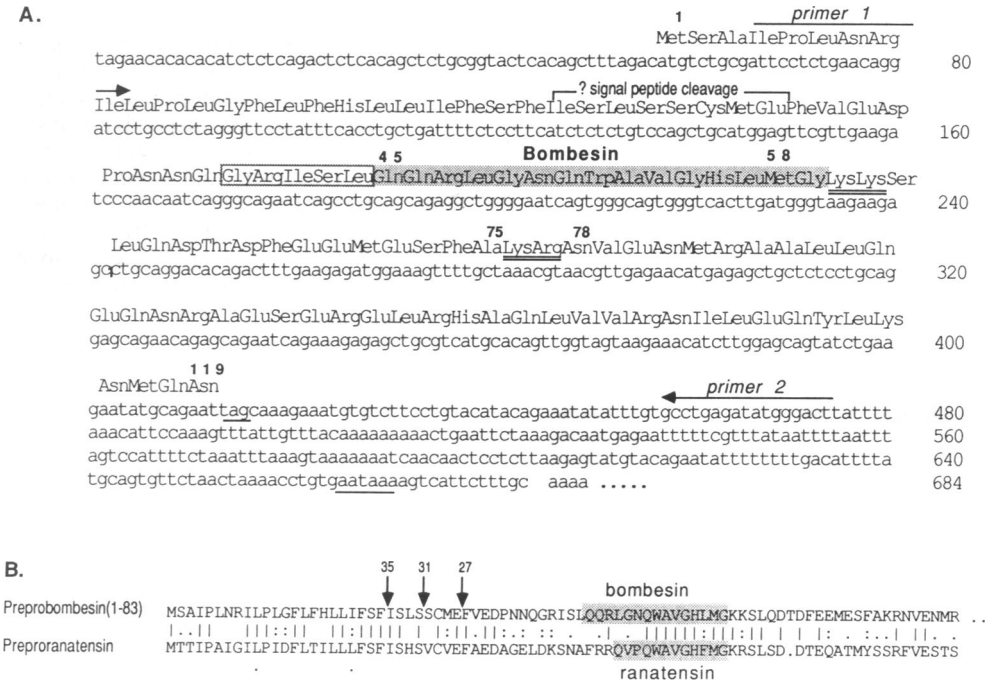

FIG. 2. (*A*) Sequence of the cDNA encoding amphibian bombesin. The sequence of cDNAs encoding amphibian bombesin was determined on four clones. Amino acids are numbered above each line and nucleotides are numbered at the end of each line. The amino acid sequence of bombesin is shaded. An amino-terminal glutamine provides the amino-terminal pyroglutamyl residue of bombesin; a carboxyl-terminal glycine provides the amide for the carboxyl-terminal methioninamide residue of bombesin. The potential chymotrypsin-like recognition sequence preceding bombesin is boxed. Dibasic cleavage points are double underlined. The stop codon and polyadenylylation signal are underlined. The region of potential signal peptide cleavage is labeled. The two oligonucleotide primers used for PCR amplification of mRNAs encoding bombesin in the stomach are indicated by the labeled horizontal arrows. (*B*) Best-fit (31) comparison of the first 83 amino acids of the bombesin prohormone and the entire amphibian (*R. pipiens*) ranatensin prohormone, showing 48% identity in an 82-amino acid overlap. Vertical arrows indicate possible sites of signal peptide cleavage and the numbers on the arrows indicate the size of the peptide that would result from concomitant cleavage and amidation at the carboxyl terminus of bombesin. A size of 35 would result if cleavage was similar to that observed for preproranatensin in *R. pipiens* skin (B.W.G., unpublished data), a size of 31 would result predicted solely on the optimum signal peptidase consensus recognition sequences (32), and a size of 27 would derive if cleavage yields a similarly sized peptide as GRP.

brain, and gut (Fig. 3*A*). As expected, the levels of bombesin mRNA in skin greatly exceeded levels in brain or gut. Treatment with norepinephrine greatly increased levels of bombesin mRNA in skin but had no effect on mRNA levels in brain or gut. To prove that the same bombesin precursor was expressed in skin and gut, RNA from skin and stomach was reverse-transcribed and amplified by PCR using primers that spanned the bombesin prohormone. The resulting fragments were then subcloned and sequenced. Primers (Fig. 2*A*) were chosen to span the entire coding region of the bombesin cDNA. This procedure resulted in identically sized amplified products from skin and stomach (Fig. 3*B*) and the DNA sequences obtained from skin and stomach subclones were identical to each other and to the sequence shown in Fig. 2*A*. Thus the same bombesin prohormone is expressed in skin and gut.

Chromatography, however, showed clear differences between BLI detected in brain and gut. Size-exclusion chromatography of skin extracts showed only a single peak of BLI in skin consistent in size with authentic bombesin (Fig. 4). By contrast, gut extracts showed two peaks, one consistent in size with bombesin and a second larger peak that was eluted in fractions similar to those containing mammalian GRP. As expected, the levels of BLI in skin were 100-fold higher than in gut. Thus processing of the bombesin precursor results in the presence of only bombesin in skin but both a bombesin-sized and a GRP-sized peptide in gut.

DISCUSSION

The tetradecapeptide bombesin was originally characterized from the skin of two *Bombina* species, *B. bombina* and *Bombina variegata*. These species are threatened and research on them is limited. *B. orientalis*, however, are readily available and have been reported by immunological evidence to contain high levels of bombesin in skin (34). By using HPLC and mass spectrometry, we have confirmed the existence of authentic bombesin in the skin of *B. orientalis*. This was done by identifying the appropriate molecular ion of m/z = 1619.9 and by identifying fragment ions of both the carboxyl and amino terminus of bombesin. Precursor forms of bombesin were not observed by this technique, but peptides larger than 30 amino acids were beyond the mass range used for molecular ion detection. Of note, ion species at m/z 2580.5, 2564.5, and 2379.4 and partial sequences consistent with the homolytic peptide bombinin (30) were identified and will be described separately (B.W.G., D. Tang, R. Mandrell, and E.R.S., unpublished results).

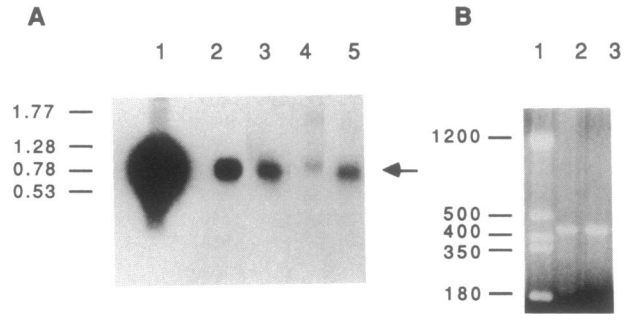

FIG. 3. (*A*) Northern blot analysis of bombesin RNA expression in *B. orientalis*. Total RNA (10 μg) or poly(A)$^+$ RNA (2.5 μg) was electrophoresed through 1.75% formaldehyde/agarose and transferred to a nylon membrane and hybridized to ^{32}P-labeled antisense bombesin cRNA probe. Lanes: 1, total RNA from norepinephrine-stimulated dorsal skin; 2, total RNA from unstimulated dorsal skin; 3, poly(A)$^+$ RNA from stomach; 4, total RNA from stomach; 5, total RNA from brain. Sizes shown are based on mobility of ethidium bromide-stained RNA size markers (data not shown). To prevent overexposure of skin RNA levels, two exposure times are shown: exposure time for lanes 1 and 2 was 18 hr and for lanes 3–5 was 72 hr. (*B*) PCR amplification of stomach and skin RNA. Poly(A)$^+$ RNA from *B. orientalis* dorsal skin or stomach was reverse-transcribed and PCR-amplified. Primers used are shown in Fig. 2*A*. Bands amplified from skin RNA (lane 2) and stomach RNA (lane 3) appeared identical in size. Size markers (lane 1) were ethidium bromide-stained *Hin*fI- digested pGem-1.

Mass spectrometric analysis of the peptide content of *B. orientalis* indicated this was a suitable species from which to clone cDNAs encoding amphibian bombesin. By using oligonucleotide probes, cDNAs were isolated and sequenced as shown in Fig. 2. The overall structure of the bombesin cDNA is similar to that of the cDNAs encoding the other known bombesin-like peptides (28). The exact cleavage site from the signal peptide cannot be determined from DNA sequence data, but potential signal peptide cleavage sites are indicated in Fig. 2*B*. As for all the other bombesin-like peptide prohormones, a dibasic amino acid cleavage sets off the carboxyl terminus of bombesin. Of note, there are no basic amino acids flanking the amino terminus of bombesin. The amino terminus is, however, flanked by a 5-amino acid sequence Gly-Arg-Ile-Ser-Leu, which is very similar to the sequence Gly-Arg-Leu-Ala-Leu that precedes the cleavage of the B peptide of rat relaxin (33), which also lacks a basic amino acid at its cleavage site. This may represent a recognition sequence of a chymotryptic-like enzyme. Within the bombesin prohormone, there is a second dibasic cleavage site allowing potential production of other peptides. Data base search (GenBank, April 1990) did not reveal homology of any of these peptides with any other known peptides. Data base search showed highest homology of the precursor at both the nucleic acid level and the amino acid level with the prohormone for amphibian ranatensin from *Rana pipiens*. As would be predicted, homology is highest in the amino-terminal portion of the prohormone. Of note, the terminal methioninamides of both bombesin and ranatensin are positioned identically, 58 amino acids from their respective translational starts (Fig. 2*B*). Interestingly, there is greater homology between amphibian proranatensin and amphibian probombesin than is found between the respective prohormones for the mammalian bombesin-like peptide GRP and amphibian bombesin. This suggests the possibility of gene conversion between the amphibian genes for bombesin and ranatensin.

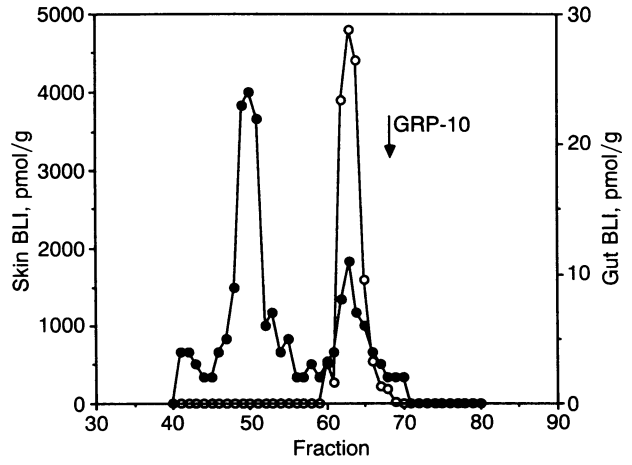

FIG. 4. Chromatography of frog skin (○) and frog gut (●) BLI. Extracts of frog gut or skin were fractionated on a 1 × 95 cm Sephadex G-50 superfine column. Fractions (1 ml) were collected and assayed for BLI. Arrow shows elution position of GRP-10. Elution of higher molecular weight peak in gut is similar to the elution position observed for GRP in other chromatography runs. Results shown are for one set of extracts. Similar results were obtained for three tissue extractions and chromatographic separations.

Richter *et al.* (35), using methods similar to those described herein, have reported the cloning of cDNAs encoding the bombesin precursor expressed in *B. variegata*. Sequence homology between these two closely related cDNAs is 95%. The bombesin prohormone sequences are identical except for the presence of three additional amino acids in the signal peptide and nine additional amino acids (numbers 83–91, Fig. 2) in the *B. orientalis* precursor. Of note, the chymotryptic-like cleavage site at the amino terminus of bombesin is conserved in both species.

The relation between BLI in frog skin and BLI in frog brain or frog gut has been unclear. Walsh *et al.* (20) found that *X. laevis*, *R. pipiens*, and *Rana catesbeiana* express two sizes of BLI in gut and brain, a large form consistent in size with GRP and smaller size consistent in size with GRP-10 or bombesin. No BLI was found in the skin of those species. Only the *Bombina* species appear to express bombesin in skin. Similar data exist for ranatensin. All frog species examined appear to express ranatensin-like peptides in brain and gut, but only *Rana* species express ranatensin in skin (17, 20). Chromatographic analysis of skin and gut extracts of *B. orientalis* yielded similar results. All BLI in *Bombina* skin extracts was eluted similarly to authentic bombesin. By contrast, in stomach the major species of BLI was larger than authentic bombesin and closer in size to mammalian GRP. This difference between gut and skin must represent processing of the same prohormone as the same bombesin-encoding RNA appears to be expressed in both brain and gut.

By analogy with the processing of preproGRP, the large form of BLI in gut may derive from cleavage of the preprohormone at the signal peptide and at the dibasic amino acids flanking the carboxyl terminus of bombesin. Thus depending on where the signal peptide of preprobombesin is cleaved, the larger form of bombesin will be between 27 and 35 amino acids in size. If preprobombesin is cleaved analogously to preproranatensin (B.W.G., unpublished data), then the large form of bombesin will be 35 amino acids (Fig. 2*B*). If preprobombesin is cleaved to give a peptide similar to GRP then the large form of bombesin will be 27 amino acids (Fig. 2*B*). Other possible signal cleavages are shown in Fig. 2*B*. Definitive determination of the site of signal peptide cleavage will require protein sequencing. The smaller form of BLI in frog gut may be authentic bombesin or, by analogy to GRP-10, it could be the carboxyl-terminal 11 amino acids of bombesin produced by tryptic cleavage at Arg-3 of bombesin.

RNA blot analysis showed highest levels of bombesin mRNA expression in dorsal skin. The size of the bombesin mRNA expressed in frog skin, brain, and gut was identical. To prove sequence identity, PCR analysis was performed. By using primers that spanned the bombesin prohormone, only a single band of similar size was primed in both skin and gut, and sequence analysis showed the identical DNA sequences. Thus amphibian bombesin and the apparent amphibian equivalent of GRP are processed from the same mRNA. The size of the bombesin RNA in *B. orientalis* is similar to that reported in *B. variegata* (35). In *B. variegata* there is also a minor 2-kilobase RNA form that did not appear to be present in *B. orientalis*.

There are clear differences between regulation of bombesin gene expression between stomach and skin. Bombesin mRNA levels are much higher in skin than in stomach, and gene expression in skin (but not gut) is stimulated by prior treatment with norepinephrine. That all species of frogs express the bombesin mRNA in stomach suggests that the necessary transcriptional factors for gut expression are common to all species, but that only few species express the needed factors for high level expression in skin. The precise mechanism that allows certain peptides to be expressed in high levels in the skin of some frogs remains to be determined.

We have now demonstrated that in frogs, preprobombesin is processed to bombesin in skin and to a GRP-sized peptide and to a bombesin-sized peptide in gut. Thus the difference in size between mammalian GRP and amphibian bombesin reflects alternate proteolytic processing and it is thus safe to conclude that GRP and GRP-10 are truly the mammalian equivalents of bombesin.

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