We report the isolation of a novel bioactive peptide, neuromedin U-23 (NmU-23), from the defensive skin secretion of the Australasian tree frog, *Litoria caerulea*. The primary structure of the peptide was established by a combination of microsequencing, mass spectrometry and site-directed antiserum immunoreactivity as SDEEQVPGPVSNGLFFPRN-amide (M, 2580.6). A synthetic replicate of frog NmU-23 displaced monoradiiodinated rat NmU-23 from uterine membranes in a dose-dependent fashion indistinguishable from nonisotopically labeled rat NmU-23. In a rat uterine smooth muscle strip preparation, synthetic frog NmU-23 produced dose-dependent contractions identical to porcine NmU-25. However, in a preparation of human urinary bladder muscle strip, the synthetic frog peptide was more potent than porcine NmU-25 in eliciting contraction and produced desensitization of the preparation to the latter peptide. This report demonstrates that the defensive skin secretion of a frog contains a novel peptide exhibiting a high degree of primary structural similarity to the endogenous vertebrate peptide, NmU, and that this frog skin analog displays biological activity in mammalian tissues.

Amphibian skin secretions have long been known as a rich source of biologically active peptides (1). Currently, in excess of 100 peptides have been structurally characterized, and these have been classified into several families based upon primary structural similarities (2). Many of these peptides have primary structures that are either identical to their endogenous mammalian counterparts (e.g. bradykinin from the skin of the European common frog, *Rana temporaria* (3)) or possess discrete regions of identity with the active sites of such (e.g. bombesin from the skin of the European frog, *Bombina bombina* (4)). A number of approaches have been adopted to identify and isolate bioactive peptides from amphibian skin on the basis of physiological tests or bioassay. For example, contractile activity of smooth muscle preparations has enabled several peptides to be identified from a large number of amphibian species (5–8). Peptide tyrosine tyrosine, synthesized primarily in the endocrine cells of the gut and in brain stem neurons (9), was isolated from a skin extract of the South American leaf frog, *Phyllomedusa bicolor*, on the basis of its antifungal activity (10). The glandular secretion of the green tree frog, *Litoria caerulea*, found in the northern territories of Australia and in New Guinea, is known to contain large quantities of peptides, only a few of which have been structurally characterized. These include a group of homologous peptides, the caerins (11), certain of which have antibacterial and antiviral properties (12); the caerulins, whose bioactivity is unknown (13); and caerulein, with physiological effects indistinguishable from CCK-8 (14). To date, the identity of other peptides within the skin secretion remains to be established.

The neuromedin U (NmU) group of peptides exhibit limited sequence similarity with other peptide families. The prototype peptides, NmU-8 and NmU-25, the former representing the C-terminal octapeptide amide of the latter, were isolated from extracts of porcine spinal cord tissues (15), and both were found to be myoactive. NmU immunoreactivity has been found throughout the peripheral and central nervous systems, with the highest concentrations in the pituitary gland, gastrointestinal tract, and nucleus accumbens (16). Primary structural information currently exists on six other members of this peptide family from the rat (17, 18), frog (19), guinea pig (20), rabbit (21), dog (22), and chicken (23). The primary structure of a putative human NmU-25 has been deduced from cDNA encoding the human NmU precursor (24). NmU peptides have been shown to be potent stimulators of uterine smooth muscle contraction in vitro (15) and to exert a marked hypertensive effect in rats when administered systemically (25). NmU-8 and NmU-25 cause potent and selective reduction of splanchnic blood flow in the dog and rat (26, 27), and NmU-8 alters ion transport in porcine jejenum by a noncholinergic, neuronal mechanism (28). A variety of motor responses have been reported to be mediated by NmU in the gastrointestinal tract of several different species (28, 29) and between longitudinal and circular muscle of particular tissues (30). NmU may also exert a direct and indirect effect on the regulation of growth, structure, and function of the adrenal cortex (31–33). We report here, for the first time, the isolation and structural character-
Frog Skin Neuromedin U-23: Characterization and Bioactivity

ization of a 23-amino acid residue NmU analog from the defensive skin secretion of an amphibian. We further demonstrate that a synthetic replicate of this peptide is biologically active in smooth muscle tissues of rat and man and that it can effectively compete with its endogenous mammalian counterparts for specific high affinity binding sites on membrane preparations from such tissues.

MATERIALS AND METHODS

Synthetic NnU-8 and NnU-25 and Lys<sup>2</sup>-NmU-8 were purchased from Peninsula Laboratories (St. Helens, United Kingdom); NmU-23 was modified and synthesized in the laboratory. Carmichael chloride (Carbachol) was supplied by Sigma (Dorset, UK). All other reagents were of analytical or HPLC grade.

NmU-8 Radioimmunoassay—Guinea pigs were immunized with porcine Lys<sup>2</sup>-NmU-8 conjugated to ovalbumin using glutaraldehyde. Animals were given primary subcutaneous immunizations of 50 µg of coupled peptide dispersed in 1 ml of Freund’s complete adjuvant and monthly boosters of 10 µg of coupled peptide dispersed in 1 ml of incomplete Freund’s adjuvant. After two booster injections, an anti-NmU antiserum was obtained from the four frogs on a monthly basis without obvious deleterious effects on the frogs over a period of 2 years.

Collection of Skin Secretions—Young adult, captive bred specimens (n = 4, snout to vent length of 8 cm) of *L. caerulea* were obtained from a local supplier. The frogs were maintained in terraria at a temperature of 24 °C with a 12 h/12 h light/dark cycle and were fed crickets twice weekly. Following a 4-week period of acclimatization, skin secretions were obtained from the frogs by gentle electrical stimulation (4-msec pulse width, 50 Hz, 5 V) using platinum electrodes placed over the paired parotoid glands. Secretions were visible after a few seconds, and 10 µl of porcine NmU-8 standard (0–500 pg/assay tube) were added to each sample. Antiserum and NmU-8 standards (or samples) were incubated for 24 h at 4 °C, with further incubation of the assay system for 24 h at 4 °C following the addition of monoradioiodinated NmU-8 tracer. Separation of bound from free counts was achieved by the addition of 1 ml of 0.05% (w/v) dextran-coated charcoal, followed by centrifugation at 11000 × g for 30 min at 4 °C. Charcoal pellets, containing free counts, were counted using a Nuclear Enterprises NE 1600 γ-counter. Under these conditions, the sensitivity of the assay was 1.5 pg of NmU-8/assay tube. Cross-reactivity of the antisera with substance P, calcitonin gene-related peptide, pancreatic polypeptide, vasoactive intestinal peptide, neuropeptide tyrosine, peptide tyrosine tyrosine, and neuropeptide phenylalanine was assessed.

Collection of Skin Secretions—Young adult, captive bred specimens (n = 4, snout to vent length of 8 cm) of *L. caerulea* were obtained from a local supplier. The frogs were maintained in terraria at a temperature of 24 °C with a 12 h/12 h light/dark cycle and were fed crickets twice weekly. Following a 4-week period of acclimatization, skin secretions were obtained from the frogs by gentle electrical stimulation (4-msec pulse width, 50 Hz, 5 V) using platinum electrodes placed over the paired parotoid glands. Secretions were visible after a few seconds, and these were washed into a glass beaker with deionized water. The presence of constant amounts of membrane protein (200 µg/ml), and bacitracin (0.1 µg/ml) to a final concentration of approximately 50 µg of protein/ml and stored at 4 °C.

Receptor Binding Assay—Female Wistar rats (200–250 g) were killed by cervical dislocation, and both ureteric horns were removed en bloc and placed in ice-cold De Jalon solution (154 µmol NaCl, 5.95 mmol NaHCO<sub>3</sub>, 5.63 mmol KCl, 0.54 µmol CaCl<sub>2</sub>H<sub>2</sub>O, 2.78 µmol glucose) equilibrated with a carbogen mixture (95% O<sub>2</sub>, 5% CO<sub>2</sub>). Each ureterine horn was removed, washed, and bathed in a Krebs solution and maintained at 37 °C. NmU-8, NnU-25, and frog NmU-23 were dissolved in fresh De Jalon solution each day and added directly to the bath. Contractions were measured isometrically using UFI force displacement transducers (Pioden Controls Ltd., Canterbury, UK). Concentration response curves were constructed by the addition of a single concentration of an agonist, followed by two 5-min washes of the tissue to a maximum contraction being achieved; the process was then repeated with a higher concentration of the agonist. Tissue responses were normalized by expression in grams of tension produced per milligram of tissue (wt weight) (g/mg). Potency values were expressed as EC<sub>50</sub> values, determined by the method of De Leen et al. (34). Results were expressed as mean values ± S.E., and significance (p value < 0.05) was tested using analysis of variance.

Human Bladder Preparation—Full thickness specimens were taken from the dome of the bladder from two cadaver donors (with consent) and immediately placed in ice-cold Krebs solution (120 mmol NaCl, 5.9 mmol KCl, 15.4 mmol NaHCO<sub>3</sub>, 1.2 mmol NaH<sub>2</sub>PO<sub>4</sub>, 2.5 mmol CaCl<sub>2</sub>, 1.2 mmol MgCl<sub>2</sub>, 11.5 mmol glucose) previously equilibrated with a carbogen mixture (97% O<sub>2</sub>, 3% CO<sub>2</sub>) to give a stable pH of 7.4. Muscle strips (two 2 × 20 mm) were excised from the dome of the bladder and the muscle bundles were dissected from the specimen using a dissection microscope and fine silk (50) ligatures tied to each end of the strips. Strips were mounted in 2 ml organ baths containing Krebs solution and maintained at 37 ± 0.5 °C with constant bubbling (97% O<sub>2</sub>, 3% CO<sub>2</sub>). After an initial application of 1 g of tension, tissue was allowed to equilibrate for 1 h, during which time the bath was constantly perfused with Krebs solution (37 °C at 1 ml/min). Strips were equilibrated for 1 h (100 µmol NmU). In order to test the integrity of the muscle and obtain a measure of the maximum contraction, NnU-8, NnU-25, and frog NnU-23 were dissolved fresh each day in Krebs solution and added directly to the bath. Agonists were applied in a noncumulative manner; however, after exposure to an agonist, the organ bath was drained and washed (2 × 5 min) as described above before being perfused at 1 ml/min with fresh, gas-equilibrated Krebs (37 °C) for 20 min. Measurement of contraction was performed as described above.

NmU Receptor Binding Assay—Female Wistar rats were killed by asphyxiation in CO<sub>2</sub>, and the ureter were rapidly dissected and immersed in liquid nitrogen. Ureter membranes were prepared by differential centrifugation, as described previously (35). Membranes were resuspended in ice-cold HEPES (50 mmol buffer (pH 7.6) containing soybean trypsin inhibitor (10 µg/ml), pepstatin (0.5 µg/ml), leupeptin (0.5 µg/ml), antipain (0.5 µg/ml), benzamidone (0.1 µg/ml), aprotonin (30 µg/ml), and bacitracin (0.1 µg/ml) to a final concentration of approximately 10 mg of protein/ml and stored at −80 °C. Protein concentration was measured by the Biuret method (36).

Synthetic rat-NnU-23 was iodinated by the chloramine T method as described previously (35). Peptide fractions were assayed for binding to uterine membranes, and the average specific activity, as determined by a specific NnU radioimmunoassay (37), was 24 Bq/fmol. The assay was performed as described by Nandha et al. (35). Briefly, incubations were carried out at 4 °C for 60 min in a final volume of 0.5 ml of binding buffer containing 50 mm Tris-HCl (pH 7.4) and aprotonin (30 µg/ml). The presence of constant amounts of membrane protein (200 µg/ml) and...
Frog Skin Neuromedin U-23: Characterization and Bioactivity

RESULTS

Peptide Isolation—NmU immunoreactivity was detected in the gel permeation chromatographic fractions of lyophilized L. caerulea skin secretion eluting in fractions suggestive of a molecular mass of less than 3 kDa (data not shown). Radioimmunoassay indicated the presence of a least 4.5 nmol of NmU immunoreactive peptide in the sample. Nonspecific binding was determined in the presence of 1 µM unlabeled rat NmU-23. Specific binding is defined as total binding minus nonspecific binding. Binding data were analyzed by nonlinear regression to determine the affinity (K_d) of binding sites using Receptor-Fit programs (Lundsoft Software, Cleveland, OH).

Structure of the purified peptide was established unequivocally as SDEEVQPGVISNGYFLFRPRN, and the observed mass of 2580.6 Da corresponded precisely with the theoretically predicted mass of the peptide with an amidated C-terminal residue (2580.8 Da). The full molar cross-reactivity of the frog NmU-23 with the amide requiring NmU antiserum employed in a radioimmunoassay confirmed the presence of this common post-translational modification. Sequence alignment of the newly identified peptide with those of the Swiss-Prot and Gen-BankTM peptide/protein sequence data bases revealed a very high degree of sequence similarity with peptides of the NmU family (Table I). Approximately 87% of the NmU immunoreactivity applied to the original chromatographic column was purified to homogeneity.

Rat Uterus Preparation—The frog NmU-23, along with porcine NmU-25 and NmU-8 produced dose-dependent contractions in rat uterine smooth muscle preparations (Fig. 2), with EC_{50} values of 1.1 ± 0.1, 1.8 ± 0.1, and 12 ± 0.6 nM, respectively. The potencies of porcine NmU-25 and frog NmU-23 were not significantly different; however, both peptides were significantly more potent than porcine NmU-8 (NmU-25 was 6.7 times and NmU-23 was 10.9 times more potent than NmU-8). The maximum contraction elicited by each peptide was not significantly different.

Human Bladder Preparation—Application of frog NmU induced contractions of human detrusor smooth muscle strips in a dose-dependent manner (n = 4) (Fig. 3). It was apparent that 10 nM frog NmU-23 produced stronger contraction, suggesting a greater potency when compared with an equimolar concentration of porcine NmU-25 (Fig. 4). Furthermore, upon reapplication of the same concentration of porcine NmU-25 following frog NmU-23, a 35.3 ± 12.1% decrease was apparent when compared with the previously evoked response (n = 4).

Receptor Binding Assay—Synthetic frog NmU-23 displaced binding of $^{125}$I-labeled rat NmU-23 from rat uterine membranes in a dose-dependent fashion indistinguishable from nonsiotopically labeled rat NmU-23 (Fig. 5). Scatchard analysis of saturation binding data produced dissociation constants (K_d) for rat NmU-23 and frog NmU-23 of 5.10 ± 2.83 and 12.72 ± 5.29 nM, respectively (n = 3).

Peptide Isolation—NmU immunoreactivity was detected in the gel permeation chromatographic fractions of lyophilized L. caerulea skin secretion eluting in fractions suggestive of a molecular mass of less than 3 kDa (data not shown). Radioimmunoassay indicated the presence of a least 4.5 nmol of NmU immunoreactive peptide in the sample. Nonspecific binding was determined in the presence of 1 µM unlabeled rat NmU-23. Specific binding is defined as total binding minus nonspecific binding. Binding data were analyzed by nonlinear regression to determine the affinity (K_d) of binding sites using Receptor-Fit programs (Lundsoft Software, Cleveland, OH).

The amino acid sequence for frog gut neuromedin U can be accessed in the Swiss-Prot Database under accession no. P20056 (18). The amino acid sequence for rabbit neuromedin U can be accessed in the Swiss-Prot Database under accession no. P34965 (20). The amino acid sequence for human neuromedin U can be accessed in the Swiss-Prot Database under accession no. P48645 (23). The amino acid sequence for rat neuromedin U can be accessed in the Swiss-Prot Database under accession no. P15760 (42). The amino acid sequence for chicken neuromedin U can be accessed in the Swiss-Prot Database under accession no. P34963 (22). The amino acid sequence for pig neuromedin U can be accessed in the Swiss-Prot Database under accession no. P34962 (21). The amino acid sequence for guinea pig neuromedin U can be accessed in the Swiss-Prot Database under accession no. P34966 (19).

Table I

| Species       | Sequence*       |
|---------------|-----------------|
| Frog skin NmU | SDEEVQPGVISNGYFLFRPRN-NH$_2$ |
| Frog (gut)    | LKP_L_G____L_R_V     |
| Human         | FRV_F_S_FR_SR_R     |
| Rat           | YKV_N*Y_G_*.AP_G_F_  |
| Chicken       | YKV_DL_GA__IQ_R_F_  |
| Pig           | FKV_F_G_IVGGPRR____  |
| Dog           | FRL_F_G_IAQVQRQ_   |
| Guinea pig    |                 |

* gaps have been introduced in the sequence to maximize homology. Identical residues.

Structure of the purified peptide was established unequivocally as SDEEVQPGVISNGYFLFRPRN, and the observed mass of 2580.6 Da corresponded precisely with the theoretically predicted mass of the peptide with an amidated C-terminal residue (2580.8 Da). The full molar cross-reactivity of the frog NmU-23 with the amide requiring NmU antiserum employed in a radioimmunoassay confirmed the presence of this common post-translational modification. Sequence alignment of the newly identified peptide with those of the Swiss-Prot and Gen-BankTM peptide/protein sequence data bases revealed a very high degree of sequence similarity with peptides of the NmU family (Table I). Approximately 87% of the NmU immunoreactivity applied to the original chromatographic column was purified to homogeneity.

**Receptor Binding Assay—Synthetic frog NmU-23 displaced binding of $^{125}$I-labeled rat NmU-23 from rat uterine membranes in a dose-dependent fashion indistinguishable from nonsiotopically labeled rat NmU-23 (Fig. 5). Scatchard analysis of saturation binding data produced dissociation constants (K_d) for rat NmU-23 and frog NmU-23 of 5.10 ± 2.83 and 12.72 ± 5.29 nM, respectively (n = 3).
DISCUSSION

The defensive skin secretions of frogs are known to contain a plethora of biologically active peptides, many of which share common primary structural features with endogenous vertebrate regulatory peptides. Here, we report the identification and structural characterization of a NmU analog from the skin secretion of a frog for the first time. This finding thus extends the list of endogenous vertebrate regulatory peptides with frog skin counterparts and vice versa. Table I illustrates the structural similarity of frog NmU-23 to other members of the NmU family, and from this, it is clear that the highest degree of similarity (70%) is shared with the C-terminal 23 amino acid residues of intestinal NmU-25 from the frog, Rana temporaria (19). Frog skin NmU-23 has the same number of amino acid residues within its sequence as rat NmU-23; however, the two analogs differ in that the frog peptide is attenuated by two N-terminal amino acid residues, whereas two amino acid deletions have apparently occurred within the central domain of the rat peptide (17, 18). Most members of the NmU family have a putative dibasic or monobasic amino acid residue cleavage site at positions 16 and 17, which in some species leads to the endogenous generation of an N-terminally truncated molecular variant containing the C-terminal active core of the peptide (15, 22, 38). Frog NmU-23 and rat NmU-23 are unique in that they lack this putative site, and therefore it is probable that they exist only as the elongated form. Conservation of Glu⁵ and Gln⁸ in the N-terminal region of porcine NmU-25 is found in the frog NmU-23, and the highly conserved Pro¹⁰ is present in all other species except chicken (23). The N-terminal region is thought to increase the potency and prolong the activity of the peptide (15), and preservation of these residues is likely to be essential for biological activity.

Fig. 2. Concentration response curves for porcine NmU-25 (filled circle) (n = 10), porcine NmU-8 (open circle) (n = 11), and frog NmU (filled square) (n = 11) on rat uterine smooth muscle preparations. Contraction is expressed as grams of tension produced per mg of tissue (wet weight).

Fig. 3. Contractile effects of carbachol (100 μM; large arrow) and frog NmU (small arrows; 0.1 nM, 1 nM, 3 nM; 10 nM, 30 nM, 100 nM, and 1 μM, respectively) on smooth muscle strips from the human detrusor.

Fig. 4. Traces showing the repeated application of porcine NmU-25 (10 nM; small arrows) on human detrusor smooth muscle and the effect of application of frog NmU (10 nM) on the subsequent contraction elicited by porcine NmU-25 (10 nM). Tissue was washed for 30 min between each application of porcine NmU-25 and frog NmU.
The quantity of NmU-23 in L. caerulea skin secretion (several nmol) is comparatively lower than previously reported levels of other peptides from this frog, such as caerulein, which is reported in quantities as great as 1.33 micromol/g of dried skin (39). This factor, however, does not diminish its significance, since biological potency is perhaps more important than absolute quantity. The concentration of this peptide in secretions is, however, significantly higher than the levels of NmU found in neuroendocrine source tissues from other species (rat spinal cord, 61.0 pmol/g (40); chicken intestine, 97.5 pmol/g (23); rabbit small intestine, 22 pmol/g (21); and pig spinal cord, 26.6 pmol/g (40)). These levels are somewhat lower, however, than those found in frog gut, which are reported to be as much as 10-fold higher but still considerably less than levels present in L. caerulea skin secretion (19). NmU immunoreactivity was not detected previously in the skin of R. temporaria (19), and we have confirmed this observation using our radioimmunoassay. It is possible that NmU, like other previously identified vertebrate regulatory peptide analogs, is of restricted distribution within frog taxa, since we have failed to detect this peptide in skin secretions from ranid, discoglossid, leptodactylid, and neotropical hylid frogs. However, further studies on larger samples of species are required to address this question in a meaningful manner.

In studies designed to determine myotropic activity, frog skin NmU-23 produced a similar contractile response to porcine NmU-25 in a rat uterine smooth muscle preparation. The frog peptide was approximately 10.9 times more potent on a molar basis when compared with porcine NmU-8. The latter peptide produced similar EC50 values to those reported previously (16 ± 5 nM (22); 46 ± 8 nM (38)). In addition, the IC50 value obtained for inhibition of monoradioiodinated rat NmU binding to rat uterine membrane preparations (60 nM) was of a similar magnitude to that previously reported (35)). However, in the absence of suitable NmU receptor antagonists, it is not possible to state unequivocally whether both peptides activate or indeed bind to the same receptor. With such qualifications in mind, the synthetic replicate of the natural frog skin peptide did displace monoradioiodinated NmU from uterine smooth muscle membranes in a dose-dependent fashion indistinguishable from nonisotopically labeled NmU-23. Previously, porcine NmU-8 has been shown to exert a concentration-dependent contractile effect on human detrusor smooth muscle strips (40), and this effect can now be extended to include both NmU-25 and frog NmU-23. In addition, the synthetic frog peptide appeared to exert almost twice the contractile effect of porcine NmU-25. The reduction in response to 10 nM porcine NmU-25, after exposure to 10 nM frog NmU-23, may be due to an increased tachyphylactic affect of frog NmU after a relatively short exposure time. It may be that frog NmU has a comparably longer half-life than porcine NmU-25, due to the absence of the dibasic processing site where cleavage to the attenuated and more labile porcine NmU-8 form is likely to reduce expression of the bioactivity.

The discovery of a novel member of the NmU family in frog skin secretions demonstrates that this molecule is of important biological function, although its actions are likely to be specific for certain tissues. For many years, a relationship between similar peptides from the gut, brain, and skin of amphibians has been known, due to the embryological origin of all of these tissues from the neural crest (41). It may be that a regulatory loop exists between the pituitary and the skin in amphibians, as suggested previously (10).

Virtually nothing is known of the physiological roles that skin peptides play in amphibian survival; however, they may be involved in antipredation, modes of reproduction, and mating behavior or involved in control in osmoregulation (42). Establishment of these functions is difficult, due of the large variety of endogenous skin compounds, which could interact together to generate complex effects not produced by a single compound alone. Subsequently, the in vitro effects of NmU may not represent the actual physiological function. Previous studies have demonstrated that the secretion of the African clawed frog, Xenopus laevis, promotes yawning and other orofacial movements in several predatory snakes, which slowed ingestion rate and facilitated escape of the frog (43, 44). Cerulein, as well as the myoactive and neuropeptides, xenopsin, were reported to be responsible for some of these antipredator actions (42). In the arboreal L. caerulea, the high content of cerulein (13), in conjunction with NmU, may elicit peripheral or neurocentral effects to the predator that protect this species from being consumed. An alternative strategy for NmU may be to act systemically on the frog itself, to promote hypertension following injury. This would benefit the frog by preventing death by rapid blood loss. These actions would be facilitated by cerulein, which supports cardiovascular function following massive hemorrhage (45, 46). It is clear that skin NmU represents a further peptide whose specific role for the amphibian has yet to be elucidated.

This report demonstrates that amphibian skin is a very valuable resource for the discovery of novel and functionally significant bioactive peptides, many of which have mammalian counterparts that themselves await discovery.

Acknowledgments—Many thanks are given to A. Klose, D. Smettan, and K. Hann for technical assistance.

REFERENCES
1. Erspamer, V., Melchiorri, P., Falconieri Erspamer, G. F., Montecucchi, P. P., and De Castiglione, R. (1985) Peptides 6, Suppl. 3, 7–12
2. Lazur, L. H., and Atilia, M. (1993) Prog. Neurobiol. 41, 473–507
3. Anastasi, A., Erspamer, V., and Bertaccini, G. (1965) Comp. Biochem. Physiol. 14, 43–52
4. Anastasi, A., Erspamer, V., and Bucci, M. (1971) Experientia (Basel) 27, 166–167.
5. Erspamer, V., Erspamer, G. F., Mazzanti, G., and Endean, R. (1984) Comp. Biochem. Physiol. C 71, 99–108
6. Erspamer, V., Erspamer, G. F., and Cej, J. M. (1986) Comp. Biochem. Physiol. C 85, 125–137
7. Rosegiani, M., Erspamer, G. F., and Severinini, C. (1988) Comp. Biochem. Physiol. C 91, 281–286
8. Rosegiani, M., Erspamer, G. F., Severinini, C., and Simmaco, M. (1989) Comp. Biochem. Physiol. C 94, 455–460
9. Pieribone, V. A., Bradin, L., Friberg, K., Dahlstrand, J., Soderberg, C., Larhammar, D., and Hukfelt, T. (1992) J. Neurosci. 12, 3361–3371
10. Mor, A., Chartrel, N., Vaudry, H., and Nicolas, P. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10295–10299
11. Stone, D. J. M., Waugh, R. J., Bowie, J. H., Wallace, J. C., and Tyler, M. J. (1993) *J. Chem. Res.* 4, 138
12. Stone, D. J. M., Bowie, J. H., Wallace, J. C., and Tyler, M. J. (1992) *J. Chem. Soc. Chem. Commun.* 17, 1224–1225
13. Waugh, R. J., Stone, D. J. M., Bowie, J. H., Wallace, J. C., and Tyler, M. J. (1993) *J. Chem. Soc. Perkin Trans.* 3, 73–76
14. Anastasi, A., Erspamer, V., and Esedae, R. (1968) *Arch. Biochem. Biophys.* 125, 57–58
15. Minamino, N., Kangawa, K., and Matsuo, H. (1985) *Biochem. Biophys. Res. Commun.* 130, 1078–1085
16. Nandha, K. A., and Bloom, S. R. (1993) *Biomed. Res. Tokyo* 14, 71–76
17. Conlon, J. M., Domin, J., Thim, L., DiMarzo, V., Moris, H. R., and Bloom, S. R. (1988) *J. Neurochem.* 51, 988–991
18. Minamino, N., Kangawa, K., Honzawa, M., and Matsuo, H. (1988) *Biochem. Biophys. Res. Commun.* 156, 355–360
19. Domin, J., Wang, Y., Spokes, R. A., Aitken, A., Parmar, K. B., Chryanthou, B. J., and Bloom, S. R. (1989) *J. Biol. Chem.* 264, 20881–20885
20. Murphy, R., Turner, C. A., Furness, J. B., Parker, L., and Giraud, A. (1991) *Regul. Pept.* 33, 191–198
21. O'Harte, F., Thim, L., and Conlon, J. M. (1991) *Regul. Pept.* 33, 191–198
22. O'Harte, F., Bockman, C. S., Abel, P. W., and Conlon, J. M. (1991) *Regul. Pept.* 33, 191–198
23. Domin, J., Nandha, K. A., Smith, D. M., and Bloom, S. R. (1993) *Endocrinology* 133, 482–486
24. Lo, G., Legon, S., Austin, C., Wallis, S., Wang, L., and Bloom, S. R. (1992) *Mol. Endocrinol.* 6, 1538–1544
25. Barthalmus, G. T., and Zielinski, W. J. (1989) *Annu. Rev. Neurosci.* 12, 957–959
26. Zielinski, W. J., and Barthalmus, G. T. (1989) *Annu. Rev. Neurosci.* 12, 957–959
27. Gardiner, S. M., Compton, A. M., Bennett, T., Domín, J., and Bloom, S. R. (1990) *Am. J. Physiol.* 258, R22–R28
28. Brown, D. B., and Quito, (1988) *Eur. J. Pharmacol.* 155, 159–162
29. Benito-Orfíla, M., Domín, J., Nandha, K. A., and Bloom, S. R. (1991) *Eur. J. Pharmacol.* 193, 329–333
30. Magri, C. A., Patacchini, R., Giuliani, S., Turini, D., Barbanti, G., Rovero, P., and Meli, A. (1996) *Br. J. Pharmacol.* 90, 186–188
31. Malendowicz, L. K., Andeis, P. G., Markowska, A., Nowak, M., Warchol, J. B., Neri, G., and Nussdorfer, G. G. (1994) *Res. Exp. Med.* 194, 69–79
32. Malendowicz, L. K., Nussdorfer, G. G., Markowska, A., Tortorella, C., Nowak, M., and Warchol, J. B. (1994) *Neuropeptides* 26, 47–53
33. Malendowicz, L. K. (1994) *Horm. Metab. Res.* 30, 374–383
34. De Lean, A., Munson, P. J., and Rodbard, D. (1975) *An. J. Physiol.* 235, E97–E102
35. Nandha, K. A., Benito-Orfíla, M. A., Smith, D. M., and Bloom, S. R. (1993) *Endocrinology* 133, 482–486
36. Gornall, A. G., Bardawill, C. J., and David, M. M. (1949) *J. Biol. Chem.* 177, 751–766
37. Domin, J., Ghatel, M. A., Chohan, P., and Bloom, S. (1987) *Peptides* 8, 779–784
38. O'Harte, F., Bockman, C. S., Zeng, W., Abel, P. W., Harvey, S., and Conlon, J. M. (1991) *Peptides* 12, 809–812
39. Esparmer, V. (1994) *Amphibian Biology: The Integument* (Heatwole, H., Barthalmus, G. T., and Heatwole, A. Y., eds) p. 231, Surrey Beatty and Sons, Chipping Norton, NC
40. Domin, J., Ghatel, M. A., Chohan, P., and Bloom, S. R. (1986) *Biochem. Biophys. Res. Commun.* 140, 1127–1134
41. Erspamer, V., Melchiorri, P., Broddardo, M., Erspamer, G. F., Falaschi, P., Improta, G., Negri, L., and Renda, T. (1981) *Peptides* 2, Suppl. 2, 7–16
42. Lo, G., Legon, S., Austin, C., Wallis, S., Wang, L., and Bloom, S. R. (1992) *Mol. Endocrinol.* 6, 1538–1544
43. Zielinski, W. J., and Barthalmus, G. T. (1988) *Pharmacol. Biochem. Behav.* 30, 957–959
44. Zielinski, W. J., and Barthalmus, G. T. (1989) *Anim. Behav.* 38, 1083–1086
45. Bertolini, A., Guarini, S., Ferrari, W., and Rompianes, E. (1986) *Neuropeptides* 8, 25–31
46. Guarini, S., Bazzani, C., Leo, L., and Bertolini, A. (1988) *Neuropeptides* 11, 69–72
Isolation, Structural Characterization, and Bioactivity of a Novel Neuromedin U Analog from the Defensive Skin Secretion of the Australasian Tree Frog, *Litoria caerulea*

Amanda L. Salmon, Anders H. Johnsen, Michael Bienert, Gordon McMurray, Kiran A. Nandha, Steve R. Bloom and Chris Shaw

*J. Biol. Chem.* 2000, 275:4549-4554.  
doi: 10.1074/jbc.275.7.4549

Access the most updated version of this article at [http://www.jbc.org/content/275/7/4549](http://www.jbc.org/content/275/7/4549)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

Supplemental material:  
[http://www.jbc.org/content/suppl/2000/05/25/275.7.4549.DC2](http://www.jbc.org/content/suppl/2000/05/25/275.7.4549.DC2)

This article cites 45 references, 5 of which can be accessed free at  
[http://www.jbc.org/content/275/7/4549.full.html#ref-list-1](http://www.jbc.org/content/275/7/4549.full.html#ref-list-1)