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Biosynthesis and degradation of peptides derived from *Xenopus laevis* prohormones

Maria Grazia GIOVANNINI, Linda POULTER, Bradford W. GIBSON and Dudley H. WILLIAMS*
University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, U.K.

Peptides present in the skin secretion of the South African frog, *Xenopus laevis*, have been analysed by fast atom bombardment mass spectrometry and h.p.l.c. in the mass range 500–3200 Da. We have investigated the effects of successive glandular secretions induced by noradrenaline injections on these peptide levels and have found that the replenishment of the whole range of peptides is complete within 2–6 days. Intact secretory vesicles free of cellular contaminants contain a relatively large number of peptides with molecular masses in the range 2400–2700 Da. We have termed these peptides primary products or spacer peptides, since they originate from spacer regions of the precursors to xenopsin and caerulein. However, if the secretory vesicles are disrupted during the collection procedure and the solution containing the secretion is kept at room temperature for up to 2 h, relatively little of the larger peptides remain. By comparing the relative levels of the various peptides present in these secretions we have found that the larger peptides are proteolytically cleaved into smaller fragments by a novel cleavage at the *N*-terminal side of a lysine residue (at Xaa–Lys bonds where Xaa is Leu, Gly, Ala or Lys). Preliminary evidence has been obtained suggesting that the larger intact peptides possess lytic activity whereas the smaller proteolytic fragments appear relatively inactive. This may represent a mechanism by which the secretions are rendered harmless to the frog itself, since prolonged exposure would be expected to result in toxic effects. The dorsal glands of *X. laevis* thus appear similar to endocrine glands, since they are involved in peptide biosynthesis, secretion and subsequent proteolytic degradation.

INTRODUCTION

The secretions obtained from the dermal glands of several species of amphibia have been shown to contain many polypeptides of different physiological activities [1,2]. The octapeptide xenopsin [3], the decapeptide caerulein [4], the tripeptide thyrotropin releasing hormone (TRH) [5], as well as an undecapeptide of unknown activity, PGL^a [6,7], have all been isolated from the skin of the South African frog, *Xenopus laevis*. In addition, more than 30 other peptides have recently been isolated and sequenced in this laboratory by a combination of f.a.b.-m.s., Edman degradation and other techniques [6; L. Poulter, B. W. Gibson & D. H. Williams, unpublished work]. Many of these latter peptides belong to the xenopsin, caerulein and PGL^a precursor families [6], as has been determined from examining the cloned cDNA sequences of these precursors [8–10]. These peptides were found to originate from the spacer regions of the precursors to xenopsin and caerulein, i.e. regions of the precursor that do not constitute the signal sequence or the originally identified hormone product. A spacer peptide is then defined as a peptide produced by proteolytic processing of a spacer region. In addition to these peptides, other peptides belonging to unknown precursor families were also found to be present in these secretions (L. Poulter, B. W. Gibson & D. H. Williams, unpublished work). Several of these peptides resemble the spacer peptides derived from the caerulein and xenopsin precursors. Preliminary evidence suggests that these larger spacer peptides are cytolytic and many possess an α-helical structure, similar to melittin [6].

The secretion of *X. laevis* represents a useful system to investigate not only the mechanisms of processing [6; L. Poulter, B. W. Gibson & D. H. Williams, unpublished work] and biosynthesis of the peptide precursors, but also the fate of the peptides after secretion. In the present paper, we have investigated the time course of peptide levels in the frog skin secretions as a function of the interval between consecutive noradrenaline injections as well as the changes in these levels after secretion, when it appears that they are further cleaved by proteolytic enzymes also present in the secretions.

METHODS

Materials

H.p.l.c. grade acetonitrile and water were purchased from Fisons (U.K.). Trifluoroacetic acid and triethylamine were distilled before use. All other reagents were of analytical grade.

Time course experiment

Male *X. laevis* frogs (15; each 50–100 g) were each injected with 0.5 ml of 10^{-4} M-noradrenaline into the subepidermal tissue and then were immersed in 100–200 ml of 50 mM-NaCl/25 mM-ammonium acetate, pH 7.0. Within a few minutes, the glands in the dorsal skin of the frogs were seen to exude a copious secretion which covered the dorsal skin. After 15 min the frog was cleaned and removed from the solution, and the solution was quickly frozen and lyophilized. Following this initial treatment ($t = 0$), the frogs were divided into five groups

Abbreviations used: f.a.b.-m.s., fast atom bombardment mass spectrometry; TRH, thyrotropin-releasing hormone.

* To whom correspondence and reprint requests should be addressed.

of three and the noradrenaline injection and collection procedure was repeated after 2 days (group N1), 6 days (group N2), 10 days (group N3), 14 days (group N4) and 25 days (group N5). The exudate obtained from each treatment was subjected to the following experimental procedure. The lyophilizate was resuspended in 2% (v/v) acetic acid and chromatographed on a column (18.5 cm × 0.8 cm) of Sephadex G-10 by elution with 2% acetic acid. Fractions (1.4 ml) were collected at a flow rate of 1.4 ml/min and the elution profile was monitored by the u.v. absorbance at 210 nm. The six fractions giving the highest u.v. absorbance were collected, combined and dried under vacuum. This portion of the lyopilizate was then redissolved (approx. 1 mg/ml) in 2% acetic acid and injected directly onto a h.p.l.c. analytical column (Spherisorb S50DS2; Phase Sep, U.K.) on a Varian 5000 h.p.l.c. system. The aqueous phase consisted of 0.1% trifluoroacetic acid in water and the organic phase consisted of 0.08% trifluoroacetic acid in acetonitrile. The peptides were eluted by a two-step linear gradient from 0 to 45% organic phase in 45 min and from 45 to 60% organic phase during the following 10 min. The elution was completed by running the system at 60% organic phase for an additional 10 min. The flow rate was 1.5 ml/min and the elution profile was monitored by u.v. absorbance at 210 nm. All the eluted peaks were collected for further analysis by f.a.b.-m.s.

Post secretion analysis

A single frog was treated with noradrenaline as described before. A 20 ml portion of the medium (control) was collected exactly 15 min after the injection and immediately frozen. The frog was then left in the solution for a total of 2 h. After removing the frog, the remaining solution was frozen. The frozen secretions were then lyophilized and treated as previously described and chromatographed onto a Spherisorb S50DS2 analytical column (Phase Sep, U.K.) in a Waters h.p.l.c. system equipped with a Cecil CE 212 variable u.v. wavelength detector. The aqueous phase consisted of 0.1% trifluoroacetic acid in water titrated to pH 3.5 with triethylamine. The organic phase consisted of 0.08% trifluoroacetic acid in acetonitrile. A linear gradient was run from 0 to 60% organic phase over a period of 60 min. Increasing the pH of the aqueous solution to pH 3.5 by the addition of triethylamine resulted in a dramatic improvement in the resolution of the later eluting peptide components. The eluted peaks were collected, lyophilized and subjected to f.a.b.-m.s. as described earlier [6].

To investigate the possibility of variation in results between frogs, three different frogs were treated with noradrenaline and kept for 2 h in the solution. The secretion was treated as described previously. The h.p.l.c./m.s. results were then compared with those obtained in a separate experiment on a further three frogs in which the exudate was frozen after 15 min.

Vesicle preparation

After injection of noradrenaline, the frog was immersed in 70–100 ml of vesicle-preparation buffer (100 mM-NaCl/50 mM-ammonium acetate, pH 7.0). The frog was removed from the solution containing the cloudy vesicle suspension exactly 5 min after the injection. The solution was then centrifuged at 10000 *g* for 10 min. The supernatant was discarded and the pellet, resuspended with the vesicle preparation buffer (50 ml for each 100 ml of starting solution), was centrifuged again. The vesicle pellet was redissolved in 2% acetic acid (10 ml for each 100 ml of starting solution) and sonicated for 2 min to disrupt any intact vesicles. This solution was frozen, lyophilized and prepared as described previously.

Identification of secreted peptides by f.a.b.-m.s.

The peptides were identified both from their h.p.l.c. elution profile and from f.a.b.-m.s. analyses as previously described [6]. Peaks eluting from the h.p.l.c. column were collected and lyophilized before f.a.b.-m.s. analysis. After resuspension in 2% acetic acid a small portion (1–5 μl) was directly dried under vacuum onto the probe tip. Approx. 2 μl of a mixture of diglycerol/thioglycerol (1:1) or dithiothreitol/dithioerythritol (3:1) was added and mixed with the sample before insertion into the ion source. The f.a.b.-m.s. spectra were taken on a Kratos MS50 equipped with a high field magnet, mass range 10000 at 8 kV. The Ion Tech f.a.b. gun was operated at 8 kV with a current of 20–30 μA with xenon as the bombarding gas. The scan speed was 100–300 s/decade over a mass range of m/z 3000–500. The protonated molecular ions $(M\mathrm{H})^+$ (positive ion mode) or deprotonated molecular ions $(M-\mathrm{H})^-$ (negative ion mode) yielded the molecular masses of the peptides by simple subtraction or addition of the mass of a proton from the $(M\mathrm{H})^+$ and $(M-\mathrm{H})^-$ ions, respectively.

RESULTS

Time course

The injection of 50 nmol of noradrenaline into the dorsal subepidermal tissue results in a copious secretion from the dorsal cutaneous glands after an initial delay of approx. 2–5 min. Under normal conditions, approx. 100 mg of crude lyophilizate was obtained from each frog after the injection of noradrenaline. However, a repeated injection of the same amount of noradrenaline 2 days after the first treatment failed to give the same response from the animals, and only a small amount of exudate (about 35 mg/frog) was obtained. At 6 days after the first injection the frogs seemed capable of responding normally to the noradrenaline stimulation and the recovery was similar to the control ($t = 0$) of about 100 mg of crude lyophilizate/frog.

A typical h.p.l.c. trace of frog secretion acid extract obtained at $t = 0$ (control) is shown in Fig. 1(*a*). The chromatogram shows a large number of peptides present at levels comparable with those of caerulein, xenopsin and PGL^a. The majority of the peptides had been previously identified [6; L. Poulter, B. W. Gibson & D. H. Williams, unpublished work] and the f.a.b.-m.s. experiments revealed the presence of peptides with molecular masses ranging from 700 to 2700 Da. Figs. 1(*b*) and 1(*c*) show the h.p.l.c. chromatograms of the acid extracts of the secretions obtained after 2 days and 6 days, respectively.

The h.p.l.c. trace ($t = 2$ days) differs from the control profile in the almost total absence of peptides. However, after 6 days, the h.p.l.c. profile shows all of the characteristic peaks to be present at almost the same level as in the control chromatogram. The h.p.l.c. trace ($t = 6$ days) was recorded at a sensitivity 2.5 times lower than

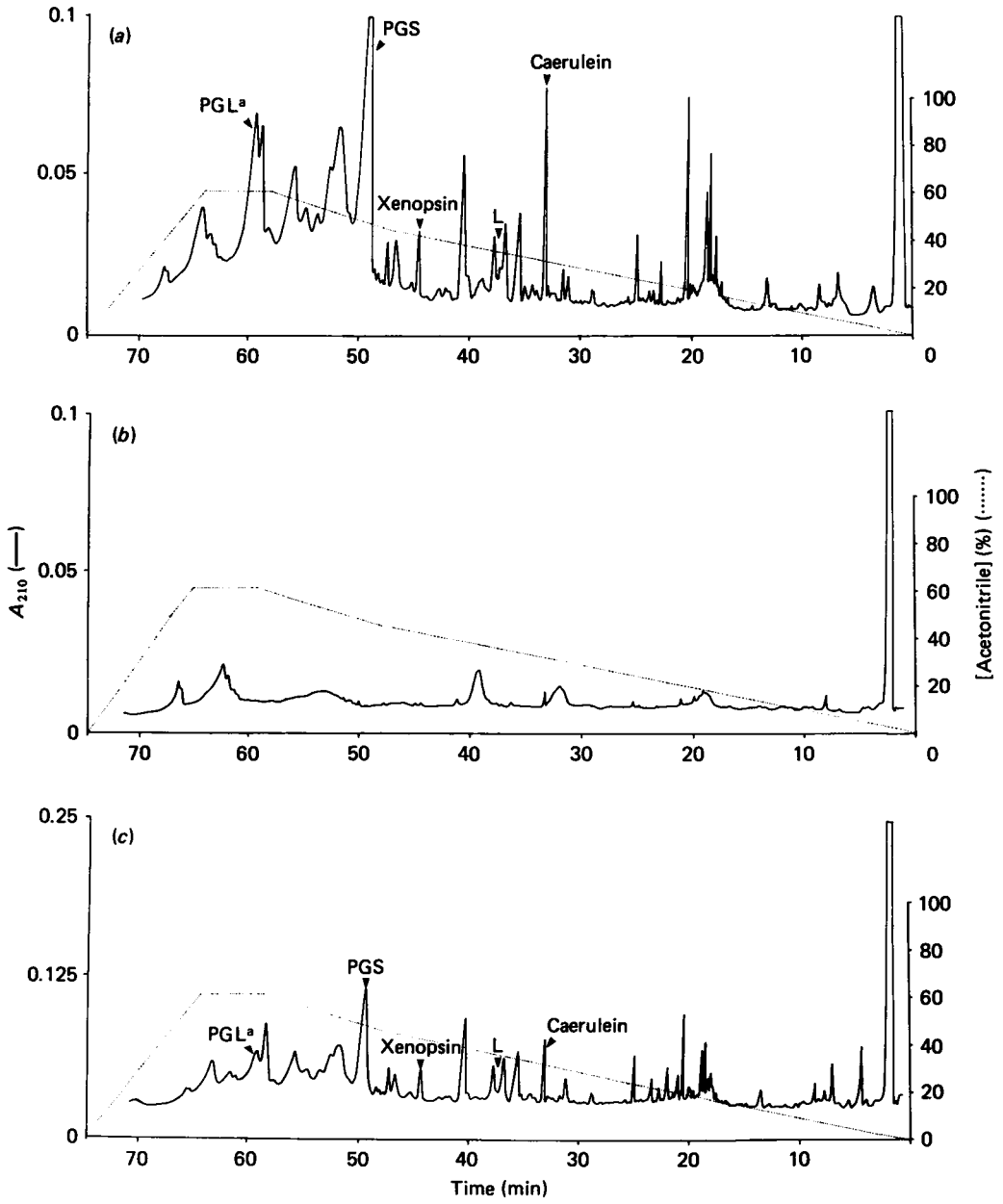

Fig. 1. H.p.l.c. traces of *X. laevis* skin secretion acid extracts

(*a*) $t = 0$ days (control); skin secretion collected after the injection of noradrenaline in previously untreated frogs. (*b*). $t = 2$ days; skin secretion collected from frogs administered with noradrenaline 2 days after the first treatment. (*c*) $t = 6$ days; skin secretion collected from frogs administered with noradrenaline 6 days after the first treatment. Trace (*c*) has been recorded at sensitivity 2.5 times lower than the preceding two. The amount of material injected was similar to the control both for the 2 day and the 6 day runs ($t = 0$ days, injected 1/65 of the crude lyophilizate, corresponding to 1.54 mg; $t = 2$ days, 1/26, corresponding to 1.38 mg; $t = 6$ days, 1/73, corresponding to 1.38 mg).

the other two, although the amount of material injected was similar. From these h.p.l.c. traces it is not possible to quantify the relative amounts of eluting peptides, both because of the complexity of the traces and because of the co-elution of many of the components. Furthermore, the enzymes responsible for the cleavage of the intact spacer peptides into subfragments seem to act immediately after secretion, presumably when the vesicles are disrupted. Hence, even a delay of a few minutes in the collecting and freezing of the secretion might cause a significant difference in the relative amounts of the primary fragments (intact spacer peptides) with respect to the subfragments.

The h.p.l.c. trace ($t = 6$ days) in Fig. 1(*c*) indicates that the system has a faster rate of recovery than the 2 weeks reported by Dockray *et al.* [12]. While the frogs appear to secrete reasonable amounts of peptides only 6 days after the depletion of the glands, it is possible that not all the glands have completely regenerated or reached their normal activity level. A comparison of the h.p.l.c. trace ($t = 6$ days) with other traces ($t = 10$, 14 and 25 days) shows little difference in profile (results not shown),

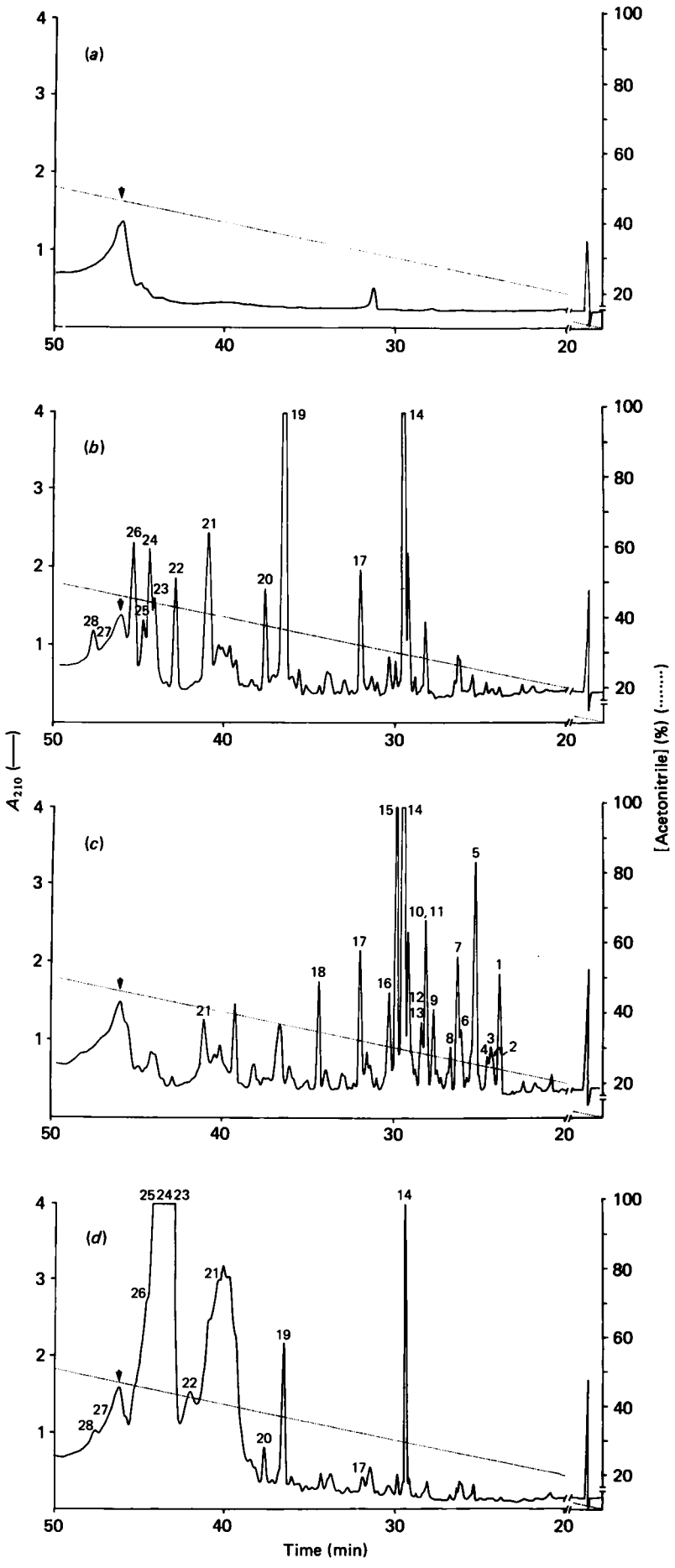
(a)
(b)
(c)
(d)
A_{210} (——)
[Acetonitrile] (%) (.....)
Time (min)
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Table 1. Sequences and M_r values of the primary products of prohormone processing

Vertical arrows indicate the major (↓) and minor (↑) sites of cleavage at Xaa–Lys bonds. Fragments originating from the cleavage are shown by horizontal arrows (←→). Numbers in parentheses refer to h.p.l.c. peaks shown in Figs. 2(*b*) and 2(*c*). Subfragments not present as major peaks in the h.p.l.c. runs are labelled with '(nd)'; however, all these subfragments have been identified by mass spectometry. Variable amino acids in the fragments originating from the same precursor are identified by a line above the single letter code.

Caerulein precursor fragments	
GFGSFLGKALKAALKIGANALGGSPQQ-OH ←(18)→ ←(1)→	M_r 2599 (24)
GFGSFLGLALKAALKIGANALGGAPQQ-OH ←(18)→ ←(4)→	M_r 2583 (25)
GLASLLGKALKAGLKIGTHFLGGAPQQ-OH ←(nd)→ ←(9)→	M_r 2644 (26)
GLASFLGKALKAGLKIGAHLLGGAPQQ-OH ←(11)→ ←(nd)→	M_r 2614 (28)
GFASFLGKALKAALKIGANMLGGAPQQ-OH ←(nd)→ ←(nd)→	M_r 2657 (27)
Xenopsin precursor fragment	
GWASKIGQTLGKIAKVGLKELTQPK-OH ←(10)→ ←(13)→ ←(nd)→ ←(nd)→	M_r 2661 (23)
Xenopsin precursor-like fragment*	
GWASKIGQTLGKIAKVGLQGLMQPK·OH ←(10)→ ←(12)→ ←(nd)→ ←(nd)→	M_r 2607 (22)
PGL[a]	
GMASKAGAIAGKIAKVALKAL-NH_2 ←(3)→ ←(7)→ ←(2)→NH_2 ←(3)→ ←(nd)→ ←(nd)→ ←(nd)→	M_r 1966 (21)
PGS	
GIGKFLHSAKKFGKAFVGEIMNS-OH ←(5)→ ←(15)→ ←(8)→	M_r 2464 (19)
[Gly[10],Lys[22]]PGS	
GIGKFLHSAGKFGKAFVGEIMKS-OH ←(6)→ ←(nd)→	M_r 2407 (20)

* Preliminary cDNA sequencing data indicate that peptide PGK (M_r 2607), although very similar in structure to peptide PGK (M_r 2661), is coded by a gene different to the xenopsin gene (A. Terry & D. H. Williams, unpublished work).

indicating that the system has undergone its greatest change within 2–6 days.

Investigation of metabolism after secretion

In order to determine the fates of peptides after secretion, a comparison was made between the h.p.l.c. traces of different secretion preparations (see Fig. 2). The h.p.l.c. trace in Fig. 2(*b*) (control) was obtained from the material collected 15 min after the injection. The h.p.l.c. trace in Fig. 2(*c*) represents the h.p.l.c. run of the material collected 2 h after the injection (about 130 mg of crude lyophilizate/frog was obtained).

The differences between the two traces are rather pronounced. In particular, the larger hydrophobic peptides eluting at 35–50% organic phase have almost totally disappeared in trace (*c*) with respect to the control, (*b*). The peptides eluting in this region are primary products of prohormone processing (intact spacer peptides), which originate by cleavage at single Arg and/or double basic residues [6; L. Poulter, B. W. Gibson & D. H. Williams, unpublished work]. From the data shown here it appears that these primary products are further cleaved to give rise to subfragments which elute at a lower percentage (22–35%) of organic phase. Because of the fast rate of proteolysis and of the co-elution of many peptides in the h.p.l.c. runs, even a semiquantitative analysis is difficult to perform for such a complex mixture. The same results were obtained in the separate experiment (see the Methods section) using two sets of three different frogs.

The structures and molecular masses of the primary products found in the vesicles preparation, together with those of the subfragments, are shown in Table 1. The arrows indicate the cleavage sites at the *N*-terminal side of Xaa–Lys residues, where Xaa is Leu, Gly, Ala or Lys.

It is noteworthy that xenopsin (pyroGlu-Gly-Lys-Arg-Pro-Trp-Ile-Leu), even though it contains the sequence Gly-Lys, is not cleaved, suggesting that the secondary structure of the molecule determines the accessibility of these residues to the protease. Furthermore, the preferential sites of cleavage are located at the *N*-terminal side of a Lys-10 or Lys-11 residue. Minor cleavages are also found at the *N*-terminal side of a Lys-15 residue in the xenopsin precursor and xenopsin precursor-like fragments and PGL[a] [6; L. Poulter, B. W. Gibson & D. H. Williams, unpublished work]. However, the fragments originating from these minor cleavages are present in very low amounts even after 2 h incubation, and are not identified in the h.p.l.c. trace but are found in the mixture analysis by f.a.b.-m.s. All the other Xaa–Lys bonds are not cleaved, giving further evidence that the secondary structure of the peptides or neighbouring

Fig. 2. Partial h.p.l.c. chromatograms of *X. laevis* skin secretion

(*a*) Blank obtained from the injection of 50 μl of 2% acetic acid into the h.p.l.c. column (Spherisorb S50DS2; Phase Sep). (*b*) Control: skin secretion collected 15 min after the injection of noradrenaline. (*c*) Skin secretion collected 2 h after the injection of noradrenaline. (*d*) Vesicles: vesicle preparation. Numbers refer to peptides listed in Table 1. The same peptides in the three different traces are identified by the same numbers. Major peaks were collected and subjected to f.a.b.-m.s. analysis as described in the Methods section. The shortened peptides are not labelled. The vertical arrows indicate a peak eluting from the h.p.l.c. column under these conditions and which does not appear to be a peptide. The amount of material injected was approximately the same for all the preparations (control, 348 μg of peptides injected, corresponding to 3.04 mg of crude lyophilizate; 2 h, 380 μg of peptides injected, corresponding to 3.12 mg of crude lyophilizate; vesicles, 363 μg of peptides injected, corresponding to 1.51 mg of vesicle crude lyophilizate. The peptide content was determined by using the method of Lowry *et al.* [11].

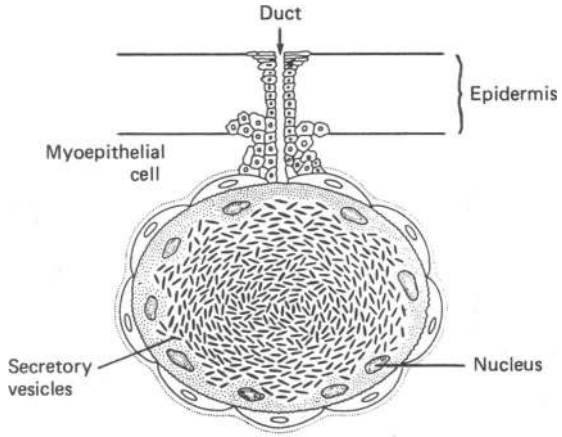

Fig. 3. Schematic representation of a granular gland of *X. laevis* (modified from [12])

amino acids play an important role in determining the accessibility of the site to proteolysis.

It was also found that several of the primary products are susceptible to dipeptidyl aminopeptidase digestion. This enzymic activity causes the cleavage of dipeptides from the *N*-terminus to give shortened peptides. The h.p.l.c. peaks of the shortened peptides are not shown in Fig. 2 due to their low levels. These latter peptides are further cleaved by the 'Xaa–Lys-specific' enzyme to give rise to subfragments in a manner similar to the processing of the primary spacer product peptides.

Vesicles

When the frogs are immersed in the vesicle preparation buffer after the injection of noradrenaline the vesicles are released into an iso-osmotic medium and therefore should remain intact [12]. The recovery of crude lyophilizate obtained from a vesicle preparation was about 20 times lower than that obtained from a non-vesicular collection, even when the frogs secreted thoroughly from the whole dorsal surface. The yield of the preparation was improved 4–5-fold by shortening the time of collection of the vesicles from 15 to 5 min. However, about 40% of the vesicles were lost during the resuspension procedure, a step necessary to remove soluble non-vesicular enzymes present in the secretion. The h.p.l.c. trace of the vesicle preparation is shown in Fig. 2(*d*).

In the vesicle preparation h.p.l.c. trace we observed the predominance of the primary products belonging to xenopsin and caerulein precursor fragment families, as well as PGS, [Gly^{10}, Lys^{22}]PGS and PGL^{a} over the subfragments. The f.a.b.-m.s. analysis of the collected fractions showed the presence of all the MH^+ values expected, indicating the presence of all the primary peptides eluting in this region (refer also to Table 1). The broader peaks observed in Fig. 2(*d*) (relative to Fig. 2*b*) are likely to be caused by overloading of the column, which was necessary to observe the less abundant peptide components. A comparison of these two chromatograms shows that there is very little cleavage of the hydrophobic primary products peptides to smaller peptides in the vesicles. However, significant cleavage has occurred within 15 min of disruption of the vesicles, as can be seen by the presence of smaller peptides subfragments in Fig. 2(*b*).

It is interesting to note that, together with the primary peptides, we also found traces of shortened peptides formed by dipeptidyl aminopeptidase digestion in the vesicles. This enzymic action on the larger spacer peptides must be very low because the majority of the peptides are present in the uncleaved form.

DISCUSSION

The release of secretory products involves disruption of the dermal glands and does not appear to be an exocytotic mechanism, but rather a holocrine secretion [12]. A schematic representation of a dorsal cutaneous gland of *X. laevis* is shown in Fig. 3 [12]. The injection of 50 nmol of noradrenaline depletes all the granular glands and it is therefore possible to synchronize the biosynthesis and replenishment of the peptides into the dorsal glands. We have used this method to understand the mechanisms which underlie the biosynthesis of the peptides in the granular glands of the skin of *X. laevis* and also to discover the time necessary for the glands to be reconstituted. Furthermore, we have tried to examine the relative amounts of the peptides originating from additional processing sites in the xenopsin, PGL^{a}, caerulein and other, as yet, unknown precursors. Our data show that the reconstitution of the dorsal dermal glands takes place between 2 and 6 days after the initial depletion. These data seem to be in agreement with the high levels of mRNA found in *X. laevis* dorsal skin after noradrenaline treatment [13]. However, we had previously noticed that the relative amount of various peptides in the total exudate extracts could change dramatically not only as a function of the time interposed between two subsequent noradrenaline injections, but also as a function of the delay between the injection and the collection of the exudate material.

It has been reported previously [14] that a haemolytic and surface-active peptide secreted by the European toad *Bombina variegata* is completely proteolysed just after 1 h at room temperature by enzymes present in the secretion. Our results obtained after leaving the secretion in the solution for 2 h at room temperature indicate that the majority of the larger primary products from *Xenopus laevis* are also rapidly proteolysed to give numerous subfragments. In particular, all the primary products belonging to caerulein precursor fragments (CPF) and to xenopsin precursor fragments (XPF) as well as the peptides PGS (M_r 2464) and [Gly^{10}, Lys^{22}]PGS (M_r 2407) (L. Poulter, B. W. Gibson & D. H. Williams, unpublished work) were completely cleaved during this time interval. On the other hand, PGL^{a} seemed to be proteolysed to a lesser extent or at a slower rate, because after 2 h about 25% of the initial amount remained uncleaved. From our data it is clear that proteolysis takes place after secretion, and it is possibly brought about by a cytoplasmic enzyme very specific for Xaa–Lys bonds [15,16], where Xaa is Ala, Lys, Leu or Gly. Alternatively, it is possible that this proteolysis could be by an enzyme packed within the vesicles, but inactive before secretion. Further studies are needed to identify, isolate and characterize this novel proteolytic enzyme.

In the h.p.l.c. trace of the pure vesicle preparation we observed the predominance of primary peptide products (M_r 2000–2700) relative to the subfragments (M_r 700–2000). We also observed the presence of all the

previously characterized peptides such as caerulein, xenopsin, PGL^a and laevitide (L. Poulter, B. W. Gibson & D. H. Williams, unpublished work).

The results obtained from our data are consistent with the following interpretation. The peptide precursors (prohormones) are processed through post-translational proteolysis inside the secretory vesicles by basic-residue-specific endopeptidases to give the primary peptides or spacer peptides [6,17,18]. The lysis of the secretory vesicles after the secretion would bring these primary products into the presence of extravesicular proteolytic enzymes capable of cleaving the primary peptides into subfragments. Cleavage of Xaa–Lys bonds, where Xaa is Ala, Leu, Lys or Gly, takes place very rapidly, but other enzymic processes, particularly dipeptidyl amino-peptidase activity, are also observed. We postulate that these processes could be more than simple non-specific degradative mechanisms; they could represent a rapid detoxification of peptides present in the secretion [18], or an 'activation' of previously inactive peptides, as is conceivable for the transformation of the peptide PGL^a-(1–11) to PGL^a-(1–10) amide [6].

The increased vesicle recovery following a shorter collection time suggested the presence of one or more factors (peptides) in the secretion that are responsible for a lytic action upon membranes. These factors would activate a cascade mechanism of disruption of the vesicular membranes and, therefore, an increase in the time before collection of the vesicles would result only in the complete disruption of the vesicles. Studies are needed to evaluate the membrane-lytic potential of some of the peptides present in the secretion and, in particular, of the primary peptide products. There are indications from the literature [19,20] that high lytic activity is found in amphiphilic peptides containing 17 or more amino acids. Some of the larger *X. laevis* peptides, when projected on an 'Edmundson Wheel' [21] clearly show an amphiphilic nature. Recently, a class of bombolitin peptides have been purified from bumblebees [20], as well as mastoparans from wasps [22] and melittin from honeybees [23]. All of these peptides are lytic on biomembranes and their biological activity has been related to their amphiphilic nature. Preliminary data indicate that some of the purified peptides have haemolytic activity as well as lytic activity on synthetic liposomes (L. Poulter, M. G. Giovaninni & D. H. Williams, unpublished work).

We cannot, however, exclude the possibility that these peptides are activated by proteolysis rather than being 'detoxified'. It has been reported that some peptides with antibiotic activity are produced by the action of proteolytic enzymes after secretion [14]. The proteolysis of PGL^a to give the subfragments PGL^a-(1–11) with subsequent *C*-terminal amidation to produce PGL^a(1–10) amide (Table 1) may represent a mechanism of physiological activation, especially since no bioactivity for the intact PGL^a has yet been found [7,8].

There is growing evidence that a single gene can code for several related neuroactive peptides [24–28] and also that a family of protein precursors can be processed to different, although related, bioactive peptides [29]. If the primary peptides originating from the spacer region of the caerulein and xenopsin precursors have predominantly lytic activity, then we will have evidence for peptides with widely differing biological functions derived from the same polypeptide precursor.

It will be interesting to determine if the secreted peptides exert their actions locally as poisons against predators [30], or if they are also released into the general circulation where they can exert their activity. It has been reported that both TRH-like peptides [31] and xenopsin-like peptides [32] are present in the skin and blood of frogs, and it has been postulated that the granular glands of the skin could be considered as endocrine glands [32]. It is possible that TRH- and xenopsin-like peptides present in blood are biosynthesized in organs other than the skin. The presence of both xenopsin with neurotensin [32] and caerulein with cholecytokinin [33] in different *X. laevis* tissues inclines us to the view that the related peptides have different activities, rather than that xenopsin and caerulein represent the amphibian counterparts of neurotensin and cholecystokinin, respectively.

Our belief is that the granular gland secretion has a defensive function, since it is produced in copious quantities when the frog is stressed and that the granular glands are reconstituted rather quickly, nearing completion within 2–6 days. Consistent with this viewpoint is that these 'cytolytic' peptides are rapidly degraded by an enzyme(s) present in the secretions. This degradation would presumably occur after the peptides have had sufficient time to exert their toxic action, most likely within the first few minutes after predatory attack.

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