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Taxonomic and Evolutionary Significance of Peptides in Amphibian Skin

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CIE, J. M. *Taxonomic and evolutionary significance of peptides in amphibian skin.* PEPTIDES **6**: Suppl. 3, 13–16, 1985.—A tentative approach to the systematic distribution of active peptides in the anuran cutaneous tissue is presented. Eleven peptide groups have been so far detected in the frog skin. The occurrence of seven major groups in different Archaeo-, Meso-, and Neo-batrachian amphibian stocks is briefly discussed. Tachykinins and caeruleins have been isolated from some Mesobatrachian *(Xenopus)* and old Neobatrachian families, even of Gondwanian ancestry, such as leptodactylids, myobatrachids, pelodryadids and ranids. A more widespread systematic distribution is reported for bradykinins and bombesins, occurring in Archaeobatrachian and Neobatrachian frogs, represented by several primitive families both of Laurasian and Gondwanian origin: among them are liopelmatids, discoglossids, myobatrachids, pelodryadids, heleophrynids and ranids. The unique position of Neotropical phyllomedusid frogs, a peculiar hylid stock with ancestral leptodactylid and myobatrachid affinities is emphasized. As many as seven major peptide groups are present in these specialized climbing anurans: tachykinins, caeruleins, bombesins, bradykinins, sauvagine, dermorphins, tryptophyllins. Phyllomedusid frogs appear to display the highest adaptive level yet reached in the peptide biochemical evolution of the amphibian skin.

Anuran skin peptides Gondwanian anurans Laurasian anurans Archaeobatrachian frogs Mesobatrachian frogs Neobatrachian frogs Phyllomedusid frogs Tachykinins Caeruleins Bradykinins Bombesins Sauvagine Dermorphins Tryptophyllins

IT has been stressed in preceding papers [2,3] that the spectra of biogenic amines occurring in the amphibian skin may vary not only among the representatives of different families, but also among those of different genera, species and subspecies. Thus, these spectra may be suitably used in biochemical taxonomy and for assessing evolutionary relationships.

This criterion has been adopted for the biogenic amines contained in toad skin [2] as well as in the skin of leptodactylid frogs [3]. It was found, for the latter, that there was a striking correspondence between amine spectra and morphological taxonomy, corroborating the validity of the biomorphological sections established for the polymorphic *Leptodactylus* genus [13, 14, 15].

The present account is intended to illustrate some preliminary data on the possible taxonomic and evolutionary significance of another set of highly active substances occurring in amphibian skin, that of peptides.

As many as 40 peptide molecules have been isolated and sequenced so far, belonging to at least 10 different families, but numerous other peptides still await isolation. Moreover, it appears likely that the relatively small molecules so far taken into consideration are synthesized within, and released from larger precursor molecules. The existence of polyproteins or prepropeptides has already been demonstrated in the *Xenopus laevis* skin for caerulein, xenopsin and thyrotropin-releasing hormone.

Data displayed in this paper reflect the present situation of the research on frog skin peptides with its gaps and uncertainties. However, this first approach to the problem which is at the root of our studies, is justified by the fact that each peptide may be actually considered a trait by which we can trace evolution of species and obtain information about the relationships existing among more or less closely related species or species groups.

The most important peptide families, more precisely those whose occurrence has been checked in a large number of amphibian species, collected all over the world, will be discussed in succession. Here it is necessary to point out that only peptide contents higher than 0.2–0.5 μg per g fresh tissue have been taken into account [10]. Below these figures bioassay is no longer reliable, owing to the unavoidable interference of disturbing contaminants.

The Tachykinins

Deca-, endeca-, and dodeca-peptide amides of the tachykinin family occur in the Neotropical leptodactylid frogs of the genus *Physalaemus*, in some Australian myobatrachids *(Uperoleia, Taudactylus* and *Pseudophryne)*, some

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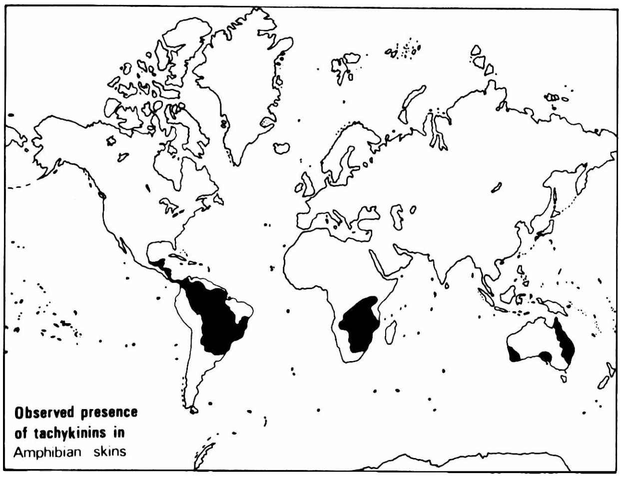

FIG. 1.

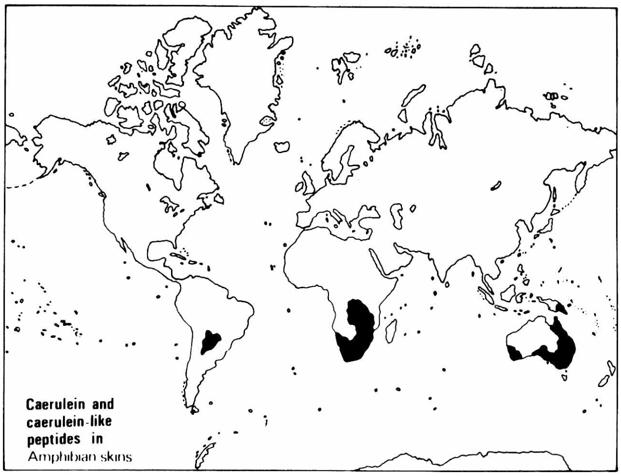

FIG. 2.

African hyperoliids *(Kassina, Hylambates)* and the very specialized Neotropical phyllomedusid stock *(Phyllomedusa, Pachymedusa, Agalychnis)*. So far, the tachykinin family is constituted by seven members: physalaemin, [Lys^5,Thr^6] physalaemin, uperolein, phyllomedusin, kassinin, [Glu,Pro^5] kassinin, hylambatin. They are often contained in the skin in remarkable amounts, as in *Physalaemus biligonigerus* (80–150 μg/g), *Ph. centralis* (70–100 μg/g) and *Hylambates maculatus* (150–200 μg/g) [8, 9, 11].

All the above anuran families have probably a common, austral or Gondwanian ancestry [16] (Fig. 1).

The Bradykinins

These peptides occur in representatives of different Archaeobatrachian and Neobatrachian families, among which are primitive liopelmatids *(Ascaphus truei)*, discoglossids *(Bombina orientalis)*, myobatrachids *(Pseudophryne)*, South African heleophrynids *(Heleophryne purcelli)*, neotropical hylids *(Phyllomedusa rhodei)* and the almost cosmopolitan super-genus *Rana* [4]. Peptides of different molecular weight are included in this family: bradykinin, [Thr^6]bradykinin, [Val^1,Thr^6]bradykinin, phyllokinin, bombinakinin-O, [Hyp^6]bradykinin, small bradykininogens [6, 8, 11]. Available evidence suggests, that, because of their generalized distribution, the bradykinins are among the phylogenetically oldest peptides thus far identified and studied.

The Caeruleins

The caerulein peptide family is composed of three members: caerulein (the prototype), [Asn^2,Leu^5]caerulein and phyllocaerulein [6,8]. Caerulein is widely distributed among the Australian pelodryadids, such as *Litoria* and *Nictimystes* [10]. As many as 25 of the 35 examined *Litoria* species contained caerulein, in some cases in exceptionally high concentrations (25–200 μg fresh skin in *Litoria caerulea, L. glandulosa, L. citropa, L. moorei, L. infrafrenata*). The content was also very high in *Nictimystes disrupta* 1000–1600 μg/g), but low in *N. tympanocryptis* and *N. vestigea* (0.2–1 μg/g). Caerulein was also well represented in some South American leptodactylids *(Leptodactylus labyrinthicus)* and African pipids *(Xenopus laevis)*. Phyllocaerulein, in turn, was isolated from *Phyllomedusa sauvagei*, a typical inhabitant of the Neotropical Chacoan scrub, and [Asn^2,Leu^5]caerulein from the African hyperoliid frog *Hylambates maculatus*. Further studies are now in progress on a fourth caerulein-like peptide traced in the skin of some Australasian ranids such as *Rana erythraea* from Taiwan and *Rana daemeli* from Queensland and Papua New Guinea. The distribution pattern of the caeruleins appears to suggest, like that of the tachykinins, a primary occurrence among southern families of Gondwanian ancestry, comprising pelodryadids, leptodactylids, phyllomedusids, hyperoliids and ranids (Fig. 2). It should be recalled that also in the case of the more primitive Mesobatrachian pipids *(Xenopus)*, a likely Gondwanian origin of their primeval lines has been claimed [16].

The Bombesins

Because of their extraordinary potency and their probable role as transmitters or modulators in the periphery and in CNS of mammals, bombesin and related peptides have attracted a great deal of attention in the last decade. Among other things, they offer the first fine example of the discovery of a peptide in the amphibian skin preceding and facilitating the discovery of an analogous peptide in the gastrointestinal tract and CNS of mammals [6, 8, 9, 11].

The bombesin peptide family may be divided into two or possibly three subfamilies: the bombesin/alytesin subfamily, the litorin/ranatensin subfamily, and the phyllolitorin subfamily.

Bombesin and alytesin, possessing a very similar sequence of 14 amino acid residues, with the C terminal tripeptide His-Leu-Met-NH_2, have been found in the skin of the Archaeobatrachian, Eurasiatic discoglossid genera *Bombina* and *Alytes*.

The peptides of the litorin/ranatensin subfamily, characterized by the C terminal tripeptide His-Phe-Met-NH_2, have been isolated from the skin of several species of pelodryadids, myobatrachids as well as from American and Australasian ranids. Very large amounts of litorin occur in *Litoria glandulosa* and *Litoria aurea* (25–80 μg/g), smaller amounts in *Nictimystes*, in myobatrachids *(Uperoleia rugosa*, 5–10 μg/g; *Pseudophryne bibroni*, 3–5 μg/g) and in Papuan ranids (*R. papua, R. krefti, R. grisea, R. arfaki, R. daemeli*, 8–12 μg/g). Ranatensins, possessing a sequence of eleven (rana tensin) or more amino acids (ranatensin C

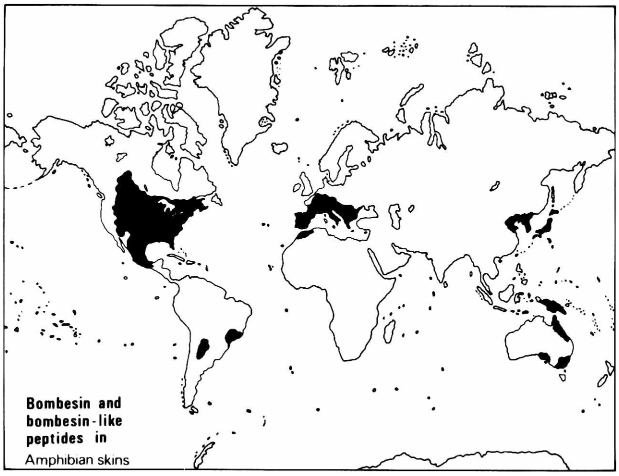

FIG. 3.

and ranatensin R) seem to be peculiar to American and Eurasian ranids.

With regard to the candidate phyllolitorin subfamily, it is presently constituted by two members, phyllolitorin and [Leu^8]phyllolitorin, characterized by the unusual C terminal tripeptide Ser-Phe(Leu)-Met-NH_2. The two peptides have been isolated from the skin of the South American *Phyllomedusa sauvagei*, belonging to the exceptional line of phyllomedusid frogs, which is certainly, as repeatedly pointed out, the anuran group most productive of peptides in the world [12]. The phyllolitorins are probably present in the skin of other phyllomedusid frogs as well, but not in the skin of *Phyllomedusa rohdei*, which contains a nonapeptide, rohdei-litorin, very similar to the mammalian neuromedin B: pGlu-Leu-Trp-Ala-Thr-Gly-His-Phe-Met-NH_2 [1].

Of considerable taxonomical interest is the fact that all the three litorin nonapeptides so far isolated from *Phyllomedusa* skin contain, unlike all other amphibian bombesins, a Leu residue at position 8 from the C terminus.

Figure 3 shows that, similarly to the bradykinins, also the bombesin peptides possess a widespread distribution in living anuran stocks, either of Gondwanian or Laurasian origin.

Sauvagine

This peptide, constituted by a 40 amino acid straight chain, occurs, in more or less conspicuous amounts in all the eleven species of phyllomedusid frogs examined, but no sauvagine has so far been traced outside the Phyllomedusinae. Particularly abundant is the peptide in *Phyllomedusa sauvagei, Ph. bicolor, Ph. burmeisteri, Ph. edentula*, less abundant in *Ph. rohdei* and other species. Sauvagine shows a close chemical and biological similarity on the one side to urotensin I, from bony fish urophysis, and on the other to the corticotropin releasing factor (CRF) from mammalian hypothalamus. Accordingly, the frog peptide displays potent peripheral actions on the vasculature and diuresis, striking effects on the anterior pituitary, with stimulation of release of ACTH and β-endorphin, and inhibition of release of GH, TSH and prolactin, finally conspicuous behavioral effects [7, 9, 11, 12].

Dermorphins

Dermorphin and [Hyp^6]dermorphin, two heptapeptides displaying an exceptionally potent peripheral and central opioid activity have been again isolated from the skin of phyllomedusid frogs (*Phyllomedusa sauvagei, Ph. rohdei, Ph. burmeisteri*). The structure of the dermorphins which is strikingly different from that of all other endogenous peptides, exhibits the unique characteristic of having a D-amino acid residue in the molecule, which is critical for their biological activity: Tyr-D-Ala-Phe-Gly-Tyr-Pre(Hyp)-Ser-NH_2 [8, 9, 12, 18].

The strange structure of the dermorphins, together with their distribution, apparently restricted to the phyllomedusid frogs, point once more to the peculiar evolutionary aspect of this amphibian stock, early diverging within the Hylidae and even showing leptodactylid-like chromosomal characters [5]. Several other uncommon adaptive trends have been reported for these slow-moving tree-frogs, such as the uricotelism, the protective wax production, a specialized osmoregulation, a unique dermal structure [20,21].

Research on the dermorphins is in its infancy. Further work will decide whether these peptides are merely a curiosity of the amphibian skin or prototypes of a new important peptide family with counterparts in mammalian tissues, especially brain and gut.

Tryptophyllins and Miscellaneous Peptides

Tryptophyllins constitute a swarm of tryptophan-containing tetra-, penta-, hepta-, decatria-peptides originally isolated from the *Phyllomedusa rohdei* skin, but probably occurring also in the skin of related *Phyllomedusa* species, as well as of other hylid frogs belonging to the South American genera *Smilisca* and *Triprion* [12,19]. It may be that a thorough study of the distribution of the tryptophyllins proves to be of taxonomical value.

Minor peptide families have not been discussed in this paper, owing to their apparently limited distribution (crinia-angiotensin I in some Australian criniid frogs) or because no studies on their actual distribution have been carried out: xenopsin from *Xenopus laevis*, granuliberin from *Rana rugosa*, TRH (thyrotropin releasing hormone) from various species of *Rana*.

CONCLUDING REMARKS

This short review on the zoological distribution of the anuran skin peptides allows us to conclude that the biosynthesis of these molecules did not, and does not now, occur at random in the different, extinct or living, phyletic lines of the amphibians.

There is no doubt that skin peptides, as genuine expressions of the work of the genetic code, will represent formidable tools in the elucidation of taxonomic and evolutionary problems.

However, an enormous amount of work remains to be done before the highly productive coding observed in amphibian skin displays its significance in the adaptive or, more generally, evolutionary processes of these creatures.

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