Brain-Gut-Skin Peptides: An Update Overview

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Summary. The authors present an overview of the main amphibian peptide families mainly derived from the skin and mostly discovered by ERSPAMER and his associates. The studies of the peptides do not only promote the understanding of their chemical, metabolic and physiological features of those molecules in amphibians, but also contribute to progress in our knowledge of the corresponding mammalian counterparts. Particular reference is made on sauvagine, tachykinin, bombesin and dermorphin families, offering new data mostly from personal contributions to this field.

It is by now common knowledge that research in the field of mammalian biologically active peptides has continuously and substantially been stimulated and enriched by that carried out on peptides extracted from the amphibian skin. There have been several occasions when the discovery of a new mammalian brain and/or gut peptide has been heralded by prior studies on the same, or a closely related, amphibian peptide. The studies on amphibian skin peptides have served to elucidate the mammalian brain-gut peptide structure. It has even been the case that a synthetic amphibian peptide has often been available several years before the corresponding mammalian molecule. This has offered the possibility to carry out extensive pharmacological studies which have proven useful for all members of a given peptide family, independent of their origin. Conversely, there have been times when such progress made on mammalian brain-gut peptides has offered suggestions useful for research on amphibian skin peptides.

Apart from a number of bioactive molecules, such as aromatic amines, (ROSEGHINI et al., 1976, 1986), at least 60 peptides have been extracted and isolated from the skins of the principal amphibian species on all continents (ERSPAMER et al., 1984, 1986). These peptides belong to thirteen distinct peptide families:

tachykinins, bradykinins, caeruleins, bombesins, crinia-angiotensins, TRH, diketopiperazines, xenopsin, sauvagine, dermorphins, tryptophyllins, pipinins/chemotactic peptides and magainins. Today we know the mammalian counterparts of the first eight of these families and we expect that the others will be found in the near future.

The present paper will update this study, referring to more recent results obtained by some of us, while directing our overall attention to only some of these peptide families.

SAUVAGINE

Sauvagine (SV) is a 40 amino acid peptide isolated from the skin of a South American frog (*Phyllomedusa sauvagei*) (ERSPAMER et al., 1980; MONTECUCCHI et al., 1979, 1980). It has two counterparts: one of these, going down to the fish, is represented by urotensin I (UI), a 41 amino acid peptide isolated from the caudal neurosecretory system of some teleosts (LEDERIS et al., 1982); the other, going up to the mammals, is represented by the CRFs, 41 amino acid peptides first isolated from the ovine hypothalamus (VALE et al., 1981). All three peptides share more than 50% of their amino acids and display almost the same biological activity.

Using a polyclonal antiserum raised in rabbits against unconjugated SV, which widely crossreacts with UI and not with CRFs, some of us have been able to show an SV/UI-like immunoreactivity (SV/UI-LI) in the caudal neurosecretory system of some fresh water teleosts (RENDA et al., 1982), in the amphibian retina (D'ESTE et al., 1983), and in some scattered immunopositive elements in the amphibian brainstem and neurohypophysis (ERSPAMER et al., 1981; ERSPAMER and MELCHIORRI, 1983).

More recently (VALLARINO et al., 1988), we have

shown the presence in dogfish (*Scyliorhinus canicula*) dorsal hypothalamus of SV/VI-LI elements, mostly localized within the *nucleus tuberculi posterioris* and in the *nucleus sacci vasculosi*. It is well known from the literature that these nuclei have no direct connections with the hypophysis, but with some other central zones such as the thalamus and other fore- and midbrain areas. Moreover, it is worthwhile to emphasize that in the same species, by using a specific antiserum against ovine CRF (VALLARINO et al., in preparation) a CRF-LI can also be shown in some other hypothalamic nuclei, such as in the preoptic nucleus that is directly connected with adenohypophysis. With these findings, it has become evident that two closely related peptides belonging to the same peptide family can be simultaneously present in the same species albeit in different zones. Identical results have been obtained very recently by GONZALES and LEDERIS (1988) by using two specific antisera against SV and CRF in some brain areas of teleosts. The functions of these two peptides in distinct, if neighbouring zones, needs to be ascertained.

Other results by using the anti SV/UI serum have recently been obtained in some marine teleost species under both normal and experimental conditions. It has been shown that a SV/UI-like peptide can intervene in some regulatory functions as a consequence of osmotic changes of the external milieu (e.g., hypotonic stress induces a dramatic increase in SV/UI-LI material) (MINNITI et al., to be published).

Moreover, using the same SV/UI antiserum, the caudal neurosecretory system of another marine species, the swordfish (*Xiphias gladius*), has also been immunohistochemically investigated. A very strong SV/UI-LI has been shown both in the characteristic big neurons (Dahlgren cells) that fill the caudal spinal cord and in their axons that, forming a conspicuous nerve bundle, reach the urophysis. Here an inextricable neurohemal network of SV/UI-LI nerve fibres concentrated particularly in the peripheral zones of that organ can be observed (Fig. 1) (MINNITI et al., in preparation).

TACHYKININS

This peptide family is formed by numerous short molecules (10/12 amino acids), and is ubiquitous at least in all animal species studied according to research carried out on some of these family members (ERSPAMER, 1981).

A useful update was given during the International Symposium on tachykinins, held in Graz (Austria)

last July, the proceedings of which were published in *Regulatory Peptides*. On that occasion, an overview was presented (NEGRI and MELCHIORRI, 1988) in amphibian skin, or, more exactly, the non-mammalian tachykinins.

Previous research (FASOLO et al., 1982; GAUDINO et al., 1985) using immunohistochemical and biochemical methods has shown the heterogeneity of the tachykinin system in the brain of the newt (*Triturus cristatus carnifex*, Laur., Urodela). More recently, employing specific polyclonal antibodies raised against hylambatin, in particular with the antiserum 1D which does not crossreact with any other known tachykinin (LAZARUS et al., in preparation) it was possible to immunostain several positive neuronal systems in the brain of two amphibians: the crested newt and the green frog (*Rana esculenta*, Anura) (FASOLO et al., in preparation). Cell bodies were stained in different regions, particularly in the ventral striatum and nucleus accumbens septi, amygdaloid complex, preoptic area, hypothalamus, tegmentum, while immunostained nerve fibres (Fig. 2a, b) were also abundant, apart from the same area, in the thalamus and the habenula. This immunoreaction was prevented by absorbing the antiserum with synthetic hylambatin, while it was unaffected by absorption with substance P. Moreover, using a double staining method, we observed a minimal overlap with the pattern shown by using a monoclonal anti substance P antiserum (CUELLO et al., 1979), which recognizes the C-terminus of the molecule.

BOMBESIN

Last year, an International Symposium on Bombesin-like peptides was held in Rome, where an exhaustive update was made. The main contributions will be soon published in the *Annals of the New York Academy of Sciences*. On this occasion a special Committee made up of the main specialists present in Rome under the chairmanship of ERSPAMER and GO, elaborated a nomenclature with the aim of comprehensively ordering the field of study. Among the recommendations made with the aim of avoiding many useless overlaps and misnomers, we would like to recall the following:

a) All peptides having C-terminal homology with frog bombesin should be indicated as "bombesin-like peptides";

b) The name "GRP" will be retained for mammalian counterparts, and all its molecular variants should be indicated with "GRP" and the first and the

Fig. 1. Caudal neurosecretory system of a swordfish stained by the "PAP" method with the antiserum to SV/ UI. **a.** Parasagittal section of caudal spinal cord showing many big immunopositive Dahlgren cells and a conspicuous bundle of immunopositive nerve axons. **b.** Dahlgren cells. **c.** Urophysis: Dahlgren cells in the medulla and neurohemal network in the cortex show strong immunostaining. Nomarski optics. a: $\times 450$, b: $\times 560$, c: $\times 90$

Fig. 2. Frog brain stained by an indirect immunofluorescent (TRITC) method with the antiserum to hylambatin. Immunoreactive nerve fibres can be observed in nucleus accumbens septi (**a**) and in septal area (**b**). $\times 300$

Fig. 3. Frog brain stained by an indirect immunofluorescent (TRITC) method with the antiserum to neuro-medin B/(litorin)-ranatensin. **a.** Immunoreactive cell bodies in the rhombencephalon. **b.** Immunofluorescent axons in the telencephalic pallium. $\times 300$

last number of the amino acid. That is the reason, for example, that "neuromedin C" will be now known as GRP (18-27).

c) Neuromedins B and U will be retained as new subclasses of peptides.

Apart from the well known functions of the bombesin-like peptides such as homeostasis promoters and autocrine growth factors for the human small-cell lung carcinomas, recent data (ANANIA et al., 1988) suggest the presence in the renal glomerular mesangium, of functional receptors for bombesin mediating proliferation and their possible implication in glomerular diseases.

Also belonging to the bombesin family is the subclass litorin/ranatensin, whose mammalian counterpart are neuromedin B, a decapeptide extracted from porcine spinal cord (MINAMINO et al., 1983). Recently, LAZARUS et al. (1988) obtained five lots of polyclonal antibodies raised against neuromedin B. Two of them displayed cross-reactivity with litorin/ranatensin while the three others did not. Only by using the cross-reacting antisera (lot NB-1C in particular) it has been possible to show immunopositive neuronal systems in the brain of the newt and the green frog (FASOLO et al., in preparation).

In the frog, cell bodies were seen in the septum, amygdala, preoptic area, tegmentum, rhombencephalon (Fig. 3a), and both in the newt and in the frog plentiful fibres (Fig. 3b) were also encountered in the septum and amygdala. Since other non crossreacting antisera are immunohistochemically ineffective, the immunostain could possibly be attributed to a ranatensin/litorin peptide. Similarly, WALSH et al. (1982), using specific antisera, observed the occurrence of both bombesin- and ranatensin-(litorin)-like peptides in the brains of some frog species.

DERMORPHINS

These are two heptapeptides amides isolated from the skin of some Phyllomedusid frogs whose structures contain the unique characteristic of a natural D-amino acid residue (D-alanine) at position two (MONTECUCCHI et al., 1981a, b). This feature is also critical for its biological activity. Moreover, the amino acid sequence of the dermorphins differs sharply from that of other known endogenous opioid peptides. Dermorphins are essentially characterized by a very potent peripheral and central opioid activity. On the basis of several investigations, it was concluded that dermorphin interacts preferentially with μ -opiate receptors but very weakly with the

δ -receptors (ERSPAMER and MELCHIORRI, 1983).

GOLDSTEIN, in a recent review (1987), affirms that the best candidate to bind selectively with μ -receptors in mammalian tissue would be "... a heretofore undiscovered mammalian counterpart of the highly μ -selective frog peptide dermorphin (of course without any D-aminoacid!)..." Of course, in our opinion this would be *with* a D-aminoacid in second position, given that [L-Ala²]-dermorphin is very inactive.

As an example of the amphibian skin as an inexhaustible source of biologically active peptides, we will very briefly mention two other recently discovered peptide families.

MAST CELL DISRUPTING AND CHEMOTACTIC PEPTIDES

This new family is represented by peptides such as Granuliberin, from *Rana rugosa* (YASUHARA et al., 1979), Pipinins, from *Rana pipiens* (HORIKAWA et al., 1985) and two Vespa-like chemotactic peptides (VSCP-1 and VSCP-5) from *Rana erytraea* (YASUHARA et al., 1986), these last two having similarities in their sequence and function to a wasp chemotactic peptide (Ves-CP-T), isolated from the venom sac of *Vespa tropica*.

Since all these peptides display a potent chemotactic and disruptive action on rat and human mast cells, it would be desirable to discover some mammalian counterpart that could be considered messengers in inflammatory and immunological processes.

MAGAININS

This new amphibian peptide family, whose name comes from the Hebrew for "shield", is composed of at least two 23 amino acid peptides that have been almost concurrently but quite independently isolated from *Xenopus laevis* both by ZASLOFF (1987) and by GIOVANNINI et al. (1987). They have been named Magainin I and II or, (Gly-10/Ly-22)-PGS and PGS respectively. The real potent lytic action on bacteria, fungi and protozoa displayed by these molecules has been, however, stressed only by ZASLOFF.

Very recently (SORAVIA et al., 1988) some other peptides have been isolated from *Xenopus* skin. Meanwhile, research in progress (MELCHIORRI, personal communication) seems to indicate that the occurrence of antimicrobial peptides is not strictly limited to *Xenopus* skin alone.

As suggested by ZASLOFF (1987), the mammalian

counterpart of magainins could exist; the gut and other natural epithelial barriers could be candidates for sites.

To conclude, it is our opinion that the amphibian skin has not yet exhausted its potential to contribute to our knowledge of bioactive peptides. The search to find the many molecules that await discovery must continue.

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