Finally, 2 µg of peptide dissolved in 50 µl of 0.1 N triethylamine bicarbonate (pH 8.5) were digested at 25 °C, overnight, by adding 10 µl of amino peptidase M solution (1 mg/ml). The enzymatic hydrolysate was directly analysed, showing the presence of an equimolar amount of aspartic acid.

The angiotensin-like radioimmunoreactivity (expressed as Ile^5-angiotensin II) varied, in skin extracts from different batches of *Crinia georgiana,* between 130 and 550 µg per g dried skin; that of skin extracts of other *Crinia* species was as follows: *Cr. glauerti,* 5–10 µg/g; *Cr. leai,* 0.8–2 µg/g; *Cr. tinnula,* < 0.1 µg/g; *Cr. pseudoinsignifera,* < 0.1 µg/g; *Cr. subinsignifera,* < 0.1 µg/g; *Cr. signifera,* < 0.1 µg/g; *Metacrinia nicholsi,* 0.9–1.0 µg/g.

Angiotensin-like immunoreactivity was probably present also in skin extracts of *Litoria adelaidensis* (0.7–0.8 µg/g dried skin), while lacking (< 10 ng/g) in numerous other Australian frog species.

Kits for Ile^5-angiotensin II were obtained from Sorin, Saluggia, Italy.

Parallel bioassay of crinia-angiotensin II and of the conventional angiotensins II is in progress. On the usual isolated smooth muscle preparations, the activity of crinia-angiotensin was similar, also from a quantitative point of view, to that of Val^5-angiotensin-II-Asp^1-β-amide (Hypertensin® Ciba).

1 Supported by grants from the Consiglio Nazionale delle Ricerche, Italy, and from the Ministry of Education, Japan.

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Occurrence of Hyp^3-bradykinin in methanol extracts of the skin of the South African leptodactylid frog *Heleophryne purcelli*[1]

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Summary. Methanol extracts of the skin of the South African amphibians belonging to the genus *Heleophryne (H. purcelli purcelli, H. purcelli depressa, H. purcelli orientalis, H. natalensis)* contain large amounts (20–500 µg/g fresh tissue) of Hyp^3-bradykinin.

Methanol extracts of the skin of the rare South African leptodactylid frog *Heleophryne purcelli* contain a bradykinin-like peptide. This has been isolated in a pure form and recognized to be Hyp^3-bradykinin:

Arg-Pro-Hyp-Gly-Phe-Ser-Pro-Phe-Arg

Materials. The fresh skins of 14 specimens of *Heleophryne purcelli depressa* and 63 specimens of *Heleophryne purcelli orientalis* collected in South Africa, Cape, during the period 1972–1975, were used in this study. The material weighed 50.8 g (average 0.66 g per fresh skin). The skins were removed from the frogs immediately after killing and extracted twice with a volume of methanol 5 times the weight of the tissue. The methanol extracts were combined and filtered and then stored in the refrigerator.

Isolation procedure. Samples of pure peptide were obtained by submitting the extracts to the following purification steps: a) washing the evaporation residue with petroleum ether in order to eliminate fat contaminants; b) passage through an alumina column which was eluted with ethanol of descending concentrations; c) chromatography on SP-Sephadex column (NH_4^+ form, 6 × 300 mm) which was eluted, in 3-ml fractions, with 120 ml of 0.15 N $HCOONH_4$, pH 6.5, and then with 30 ml of 1 N $HCOONH_4$, pH 6.5. The activity appeared at the top of 1 N ammonium formiate elution (fractions 41–46). Activity was followed by bioassay (guinea-pig ileum and rat uterus preparations). The final yield in pure peptide was approximately 3×10^{-7} moles, i.e. 300–350 µg per g fresh tissue.

Structure. Amino acid composition, after the usual acid hydrolysis, was as follows: Arg 2, Hyp 1, Pro 2, Ser 1, Gly 1 and Phe 2. It may be seen that the composition was exactly the same as that of bradykinin, with the sole exception of a hydroxyproline residue replacing a proline residue. The R_f value of the dansyl derivative of *Heleophryne* bradykinin, as compared to that of bradykinin, is slightly lower in silica gel H TLC, using the solvent system iso-propanol:methyl acetate:28% ammonia (9:7:4).

The dansylated peptide was not attacked by trypsin, but it cleaved following chymotrypsin digestion (20 µg peptide in 50 µl 0.1 triethylamine bicarbonate buffer at pH 8.2 plus 10 µl of a 0.1% chymotrypsin solution) giving origin to 3 dansylated fragments. 2 of them corresponded to DNS-Ser-Pro-Phe- and DNS-Arg, respectively. The 3rd dansylated fragment, which did not correspond to DNS-Arg-Pro-Pro-Gly-Phe, was purified and its amino acid composition determined in an amino acid analyser. The fragment appeared to be DNS-Arg(Pro,Hyp,Gly)Phe. Finally, 40 µg of the pure peptide was analysed by the dansyl Edman procedure with results shown below:

Arg-Pro-Hyp-Gly-Phe-Ser-Pro-Phe-Arg

Hyp^3-bradykinin was present in all examined species and subspecies of *Heleophryne.* However, whereas in methanol extracts of different batches of *H. purcelli purcelli, H. purcelli depressa* and *H. purcelli orientalis* it varied between 200 and 500 µg/g (expressed as bradykinin, rat uterus preparation) in extracts of *H. natalensis* it was consistently lower, barely attaining 15–25 µg/g fresh skin.

Crude extracts of *H. purcelli depressa* skin contained 150 µg 5-HT base/g tissue; extracts of *H. purcelli orientalis* 360 µg/g. The amine was separated from Hyp^3-bradykinin already during passage of the crude extract through alumina column.

It is worth remembering that the nonapeptide Hyp^3-bradykinin is included in the molecule of Vespakinin-M, Gly-(Hyp^3-bradykinin)-Ile-Asp, isolated by Kishimura et al.[2] from the venom apparatus of *Vespa mandarina.*

1 Supported by grants from the Consiglio Nazionale delle Ricerche, Italy, and from the Ministry of Education, Japan.

2 H. Kishimura, T. Yasuhara, H. Yoshida and T. Nakajima, Chem. pharm. Bull. *24,* 2896 (1976).