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Occurrence of Bradykinin-Like Substances in the Amphibian Skin¹

Besides being an enormous store-house of indole-alkylamines, phenylalkylamines and imidazolealkylamines, the amphibian skin appears to be an important production and/or storage site of highly active polypeptides.

One of them, physalaemin, has been described in a preceding communication². In this preliminary report, the occurrence of bradykinin-like substances will be briefly described. The term bradykinin-like should be interpreted in a broad sense.

So far about eighty amphibian species, collected in all parts of the world, but especially in South America, have been studied. High, and sometimes enormous, amounts of bradykinin-like compounds appeared to be present in methanol extracts of the skin of the examined *Phyllomedusae* (*Phyl. sawagi*, *Phyl. rhodei*) and *Ranae* (*R. esculenta*, *R. temporaria*, *R. pipiens*, *R. warschewitschii*, *R. japonica*, *R. catesbiana*, *R. nigromaculata*).

The polypeptide nature of the active skin constituents is shown by their rapid and complete inactivation produced by chymotrypsin incubation, and by some preliminary results obtained following acid hydrolysis of the eluates of active paper chromatographic spots.

The accompanying Table presents the activity, expressed in terms of pure bradykinin, of crude methanol extracts of the *Phyllomedusa* and *Rana* species examined. It should be emphasized that the figures are merely indicative of the relative potency of action possessed by the crude skin extracts on the different test-objects. Bradykinin served only as a standard substance, and very often the response to skin extracts was even qualitatively different from that given to bradykinin. In reality, all or nearly all aspects of the biological activity of crude *Phyllomedusa* or *Rana* extracts are due to a more or less complex mixture of active polypeptides. To elucidate the composition of this mixture, each extract should be studied separately and singularly.

Activity of crude extracts of amphibian skin expressed as bradykinin (μg per g tissue)

	Dog blood pressure	Rat uterus	Guinea-pig ileum	Dog large intestine	Rabbit large intestine
<i>Phyllomedusa sauvaigi</i> 1962 (dry skin)	800	40-90	75-200	150-200	—
<i>Phyllomedusa sauvaigi</i> 1962 (wet skin)	1200	20-50	50-150	50-100	—
<i>Phyllomedusa rhodeti</i> 1962 (dry skin)	1500-2000	250-350	500-650	300-450	—
<i>Rana esculenta</i> 1961 (wet skin)	—	7-20	15-20	15-30	300-400
<i>Rana temporaria</i> 1961 (wet skin)	—	90-100	100-130	100-130	—
<i>Rana pipiens</i> 1962 (dry skin)	40	5-10	100	—	—
<i>Rana warschewitschii</i> 1962 (dry skin)	60	70	50	—	> 100
<i>Rana japonica</i> 1962 (dry skin)	—	10	< 1.5	—	> 100
<i>Rana catesbiana</i> 1962 (dry skin)	—	2.5	< 1.5	—	> 100
<i>Rana nigromaculata</i> 1962 (dry skin)	—	2	1.6	—	> 100

So far, a partial separation of the active constituents of crude skin extracts has been carried out for *Phyllomedusa sawvagi* and *Phyllomedusa rhodei*.

In a typical experiment, the residue left by evaporation of the methanol extract of 6 g fresh skin of *Phyllomedusa sawvagi* was dissolved in 95% ethanol and absorbed on an alkaline alumina column. Elution was carried out with descending concentrations of ethanol. At least three peaks of activity appeared in the eluates: the first in 70% ethanol (polypeptide A), the second in 60% ethanol (polypeptide B), and the third in 50–40% ethanol (polypeptide C).

Polypeptide A was the one that most strictly resembled bradykinin in its pharmacological properties. However, its identity with bradykinin is improbable.

Polypeptide B, perhaps the most interesting of the series, possessed a powerful hypotensive action in the dog and the rabbit. The fall of blood pressure which could be obtained with very low doses of the polypeptide, lasted considerably longer than that caused by bradykinin, substance P, eledoisin or physalaemin doses which produced a pressure fall of the same intensity.

Polypeptide C was particularly active on the guinea-pig ileum but the shape of the contraction curve differed sharply from that produced by bradykinin.

Polypeptides identical or similar to polypeptide A have been found in *Phyllomedusa rhodei*, *Rana esculenta* and *Rana temporaria*.

Research is in progress, intended to separate the different bradykinin-like polypeptides which have already been identified and which will be identified in the skin of the new amphibian species which we are collecting throughout the world, to determine their origin and significance, to subject the different polypeptides to a thorough pharmacological study, and finally to isolate the most abundant of them in a pure form in order to elucidate their chemical structure³.

Riassunto. Gli estratti di pelle di alcuni comuni anfibi nostrani (*Rana esculenta*, *Rana temporaria*) e, ancor più, di certi anfibi sudamericani del genere *Phyllomedusa* contengono rilevanti quantitativi di polipeptidi bradichinino-simili. Negli estratti di *Phyllomedusa sawvagi* ne sono stati identificati almeno tre, a mezzo di una semplice cromatografia su colonna di allumina basica: polipeptide A, assai simile alla bradichinina per le sue proprietà farmacologiche; polipeptide B, dotato di prolungata ed

intensa azione ipotensiva; polipeptide C, particolarmente attivo sull'ileo di cavia. Sono in corso ricerche dirette all'isolamento di questi polipeptidi e al chiarimento della loro struttura chimica.

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² V. ERSPAMER, G. BERTACCINI, and J. M. CEI, *Exper.* 18, 562 (1962).

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