Pharmacological observations on phyllomedusin

Phyllomedusin is a natural decapeptide recently isolated from methanol extracts of the skin of *Phyllomedusa bicolor*, an hylid frog from the Amazonian region (Anastasi & Erspamer Falconieri, 1970).

The formulae reported below show the strict chemical resemblance existing between phyllomedusin, physalaemin from the skin of the South American amphibian *Physalaemus fuscumaculatus* (Anastasi, Erspamer & Cei, 1964), and eledoisin from the posterior salivary glands of the Mediterranean octopod *Eledone moschata* (Anastasi & Erspamer, 1963).

Pyr–Asn–Pro–Asn–Arg–Phe–Ile–Gly–Leu–Met–NH_2	Phyllomedusin
Pyr–Ala–Asp–Pro–Asn–Lys–Phe–Tyr–Gly–Leu–Met–NH_2	Physalaemin
Pyr–Pro–Ser–Lys–Asp–Ala–Phe–Ile–Gly–Leu–Met–NH_2	Eledoisin

Pure natural phyllomedusin was assayed biologically, in parallel with physalaemin, on a number of test preparations. The results are shown in Table 1.

Like that of the other tachykinins (physalaemin, eledoisin, substance P), the activity of phyllomedusin was destroyed by incubation with both chymotrypsin and trypsin (Bertaccini, Cei & Erspamer, 1965).

It may be seen from Table 1 that the activity ratio physalaemin : phyllomedusin varied not only for the different test preparations but also within the preparations; not infrequently it varied even for the same preparation during the course of an experiment.

Data reported in Table 1 reflect only the intensity of the actions displayed by the two polypeptides. Other distinctive features were represented by the duration of the fall of blood pressure, by the rapidity of relaxation of the smooth muscle after washing, and finally by the shape of the smooth muscle contraction curve.

The fresh skins which served for the preparation of pure phyllomedusin contained approximately 1100 μg of the pure peptide per g fresh tissue. The dry skin of a single large specimen of *Phyllomedusa bicolor* captured at Leticia (Colombian Amazonas) contained as much as 3500–4500 μg of phyllomedusin per g tissue.

In addition to phyllomedusin, *Phyllomedusa bicolor* contains in its skin conspicuous amounts of caerulein (probably phyllocaerulein), of phyllokinin, and of other active peptides.

It has been repeatedly pointed out that physalaemin-like peptides occur in the skin of several other *Phyllomedusa* species (Bertaccini & others, 1965). Whether these peptides are identical or not with phyllomedusin is a problem which must be solved separately in the individual species.

Table 1. *The result of parallel bioassay of physalaemin and phyllomedusin on nine test preparations.* The activity of physalaemin was always considered equal to 100, that of phyllomedusin was expressed in per cent.

Test preparation	Phyllomedusin activity (in %) (relative to physalaemin = 100%)
Dog blood pressure	40–70
Rabbit large intestine	60–150
Rabbit duodenum	30–80
Rat duodenum	70–150
Rat colon	100–150
Guinea-pig ileum	30–80
Rat oestrous uterus	150–200
Rabbit uterus	100–350
Rat salivary secretion	100–200

This investigation was supported by grants from the Italian Research Council, Rome.

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March 6, 1970.

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A simple method for chronic measurement of the electrocardiogram and blood pressure in the conscious rat

The cardiovascular responses to drugs and toxic chemicals may be markedly different in conscious animals and anaesthetized animals. Electrocardiograms (ECG) are difficult to obtain from the conscious rat without training (Farmer & Levy, 1968) or restraint (Fujita & Tedeschi, 1968). The procedure described permits implantation of electrodes which can be used for several months to monitor ECG. With slight modifications, arterial and venous cannulas have also been used for up to 2 weeks.

The mounting device (Fig. 1) consisted of a small curved polyethylene "saddle" approximately 3 × 2 cm which was cut from the side of a standard 16 oz polyethylene reagent bottle. Two electrode leads, about 10 cm in length and consisting of No 30 Teflon coated 7 strand stainless steel wire, were woven through 6 holes in the saddle leaving about 8 cm on one side for attachment to the sternum of the rat and 2 cm on the other side for connection to the electrocardiograph. Approximately 4 mm of the short lead was stripped of Teflon insulation and soldered so as to leave a small solder blob. If required, silicone rubber cannulas were also passed through additional holes in the same "saddle".

Under ether anaesthesia, two 1 cm long skin incisions were made over the anterior and posterior ends of the sternum. A 4 cm dorsal midline incision was made beginning at about the 4th vertebrae and proceeding posteriorly. The skin adjacent to these incisions was dissected free from subcutaneous tissue. The electrode leads were passed under the skin from the dorsal incision to the incisions over the sternum. The end of the electrode wires were then passed through the cartilage at each end of the sternum, using a small curved needle, and secured by twisting around the main electrode lead in the same way as standard electrical connections are made. The saddle was gently manipulated beneath the skin through the dorsal incision and the skin sutured over the saddle leaving the short connecting parts of the electrodes protruding. About 20 min was required to complete this procedure. When required, silicone rubber cannulas filled with heparinized saline, were passed under the skin and implanted in the carotid artery and jugular vein in a similar manner.