

Z. klin. Chem. u. klin. Biochem.  
7. Jg., S. 464—466, September 1969

## Contributions to the Evolution of Blood Pressure Regulation

### *Part II: Evidence for the Absence of Kinin-like Polypeptides Released by Proteolytic Enzymes for Blood Pressure Regulation in Fish*

By ROSMARIE VOGEL, H. SCHIEVELBEIN, W. LORENZ and E. WERLE, with the assistance of A. SCHMAL

From the *Institut für Klinische Chemie und Klinische Biochemie der Universität München (Direktor: Prof. Dr. Dr. E. Werle)*

(Eingegangen am 9. Mai 1969)

The intravenous injection of Padutin, bradykinin, kallidin and eledoisin is without effect on the blood pressure of cartilagenous and teleost fish. Preparations of fish pancreas and fish serum prepared in the same manner as organs of mammals and birds for prekallikrein/kallikrein or kininogen/kinin were also without effect. Therefore in fish the existence of a kallikrein-kinin like circulation regulating system can be excluded.

Die intravenöse Injektion von Padutin, Bradykinin, Kallidin und Eledoisin hat keine Wirkung auf den Blutdruck bei Knorpel- und Knochenfischen (Katzenhai und Wels). Auch Präparate aus Fischpankreas und Fischserum, die gleichermaßen wie Säugetier- und Vögelorgane aufgearbeitet waren und demnach Prækallikrein bzw. Kallikrein oder Kininogen bzw. Kinin enthalten müßten, waren ebenfalls wirkungslos. Die Existenz eines blutdruckwirksamen, Kallikrein-Kinin-ähnlichen Systems kann demnach bei Fischen ausgeschlossen werden.

In the first part of this paper we described the presence of an adrenergic system in cartilagenous and teleost fish (1).

In mammals and birds in addition to the adrenergic system for blood pressure regulation, there exist two different enzymic regulation mechanisms, which are independent of each other. These systems are the renin-angiotensin system and the kallikrein-kinin system. Phylogenetic investigations are described for the renin-angiotensin system especially with regard to the releasing enzyme (2, 3). Investigations with regard to the kallikrein-kinin system led to the detection of a kallikrein inhibitor in fish serum (4). The components of the kallikrein-kinin system are very species-specific (5, 6), with respect to the releasing enzyme (kininogenase) and also to the pharmacological effect of the kinin.

In this paper we describe investigations to find evidence for the presence or absence of the kallikrein-kinin system in fish, using methods already proved for the differentiation of the components of the system.

#### Methods and materials

The methods used for measuring the blood pressure in fish are the same as described in part I of this paper (1). As a representative of the cartilagenous fish we used the catshark (*Scyliorhinus stellaris*) and as a representative of the teleost fishes the catfish (*Silurus glanis*).

Substances and drugs used: adrenaline (Suprarenin Hoechst), noradrenaline (Arterenol, noradrenaline hydrochloride Hoechst), histamine (Imido, Roche), acetylcholine (Roche), bradykinin synth. (Sandoz), kallidin synth. (Sandoz), eledoisin (Sandoz), trypsin (Worthington), polyvalent proteinase inhibitor (Trasyolol, Bayer), renin (isorenin preparation according to (7)), Val-5-angiotensin I (Ciba), Val-5-angiotensin II (Ciba), hog pancreas kallikrein (Padutin, Bayer).

Preparation of a kallikrein-like component from fish: pancreas homogenates from shark, catfish and tench (*finca vulgaris*) were prepared by the homogenisation of one part tissue with 100 parts water. The extracts were used as such, or first separated into supernatant and precipitate, or first dialyzed for 15 hours against

fresh water. Acetone — dried powder was prepared by mixing one part of pancreas homogenate with one part of acetone; drying was performed at room temperature.

Preparation of a kinin-like component from fish: serum from fish was incubated with fish pancreas homogenate or trypsin according to known methods (5).

The sensitivity of the test animals to vasoactive substances was always tested with adrenaline and histamine respectively (fig. 1).

#### Results

*Kallikrein of mammalian origin* (2—20 units/kg body weight) had no influence on the blood pressure of fish. (One tenth of the applied minimal dose lowers the blood pressure of the dog to approximately 40% of the normal value).

*Fish pancreas* homogenate (corresponding to 2—20 mg tissue applicated/kg) induced an irreversible dose-independent decrease in blood pressure of 2—3 mmHg (see fig. 1). This slow response could not be eliminated by boiling or incubation of the homogenate with Trasyolol; it is caused by the sedimentable fraction of the homogenate. On the other hand, the depressor effect cannot be demonstrated, following the application of known treatments for the enrichment or activation of kallikrein, e.g. dialysis or acetone precipitation of the homogenates. Occasionally a pressor effect was observed (see fig. 2). The application of *trypsin* led in the fishes to a slow decrease in blood pressure. The irreversible decrease to minimal pressure resembled the circulatory failure in mammals induced by shock.

*Kallidin, bradykinin and eledoisin* had no effect on the blood pressure of fish up to doses of 20 µg/kg. (In mammals 1/100 part of this dose is effective.)

Fish serum prepared for the identification of kininogen-kinin mechanism (see methods) had no depressor effect in circulation of fish. On the contrary, a pressor effect was observed (see fig. 1).

*Renin* in a dosage of 5 mU or 50 Goldblatt-U/kg (for the preparation, see methods) had no effect on the blood

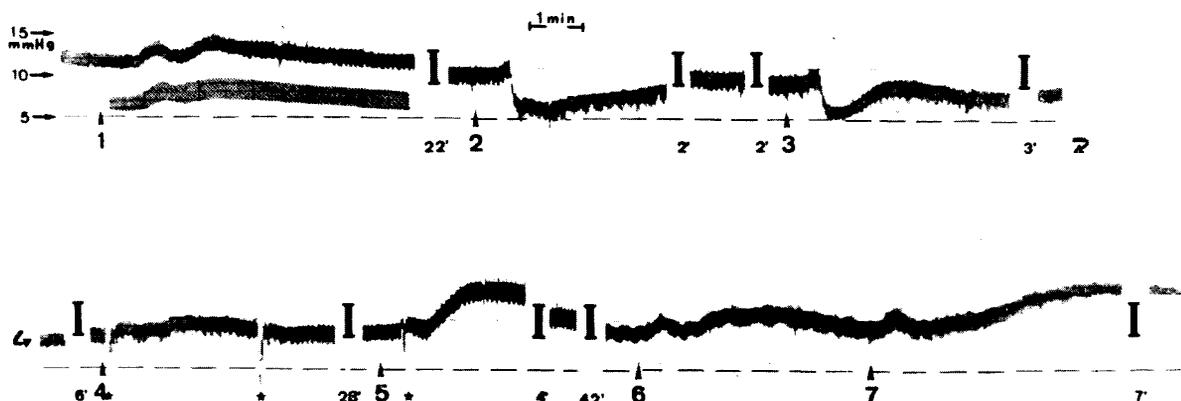


Fig. 1

Absence of components of the kallikrein-kinin system in fish

Catfish, 1,370 g; urethane anaesthesia; blood pressure in the caudal artery registered with Statham transducer (see methods in SCHIEVELBEIN and coworkers (1))

Injections in the caudal vein:

- |   |  |
|---|--|
| 1: 3.6 mg/kg pancreas of catfish homogenized 1:100 (raster: control volume) | 5: 3.6 $\mu$ g/kg noradrenaline  |
| 2: 3.6 $\mu$ g/kg acetylcholine   | 6: 0.7 ml/kg tench serum incubated with homogenate from catfish pancreas |
| 3: 3.6 $\mu$ g/kg histamine   | 7: five-fold dose of 6   |
| 4: 7.3 $\mu$ g/kg bradykinin (* spontaneous movement)                       |  |

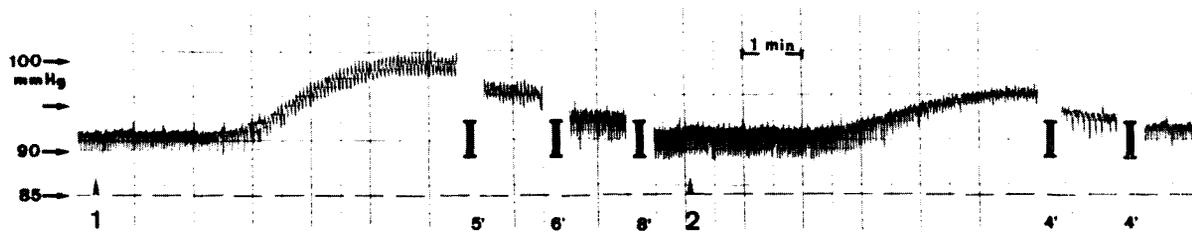


Fig. 2

Absence of kallikrein in fish pancreas

Catfish, 4,000 g; urethane anaesthesia; blood pressure in the caudal artery registered with Statham transducer (see methods in SCHIEVELBEIN and coworkers (1))

- Injections in the caudal vein:
- |  |
|--|
| 1: 5 $\mu$ g/kg adrenaline   |
| 2: Acetone-dried powder from catfish pancreas corresponding to 125 mg fresh tissue/kg body weight fish |

pressure in the species used; *angiotensin I* (10–20  $\mu$ g/kg) was also ineffective. *Angiotensin II* (7–20  $\mu$ g/kg) led to a prolonged stimulation of respiration similar to the effect of great doses of nicotine.

## Discussion

All methods known to us (5) failed to demonstrate the existence of the kallikrein-kinin system or components of this system in cartilaginous and teleost fish. The only known components are the fish serum inhibitor, which was detected by WERLE and coworkers, 1950 (4) and a kininase found by ERDÖS and coworkers (8). But the inhibitor may be an unspecific proteinase inhibitor for the regulation of proteolytic reactions known in species of lower orders (9, 10) and the kininase may be an unspecific carboxypeptidase. The observed depressor effect of pancreas homogenate cannot be attributed to a kininogenase (kallikrein) because after enrichment of high molecular components this effect was no longer observed.

Angiotensin I and II and a mammalian releasing enzyme had no effect on the blood pressure. We did not investi-

gate the possible existence of a species-specific renin-angiotensin system; evidence was obtained, however, for the liberation of pressor substances referred to by WEICHERT (2) and MALVIN and VANDER (3).

There can be several causes of the observed retardation of responses and the different dose-response relationship in fish as compared to mammals. One of these reasons may be the occurrence of analogues of derivatives of catecholamines (SCHIEVELBEIN and coworkers (1)). Finally the special anatomical conditions of the fishes can be involved. According to ROMER (11), in fish the so-called renal-hepato circulation with a widespread capillary system interrupts the caudal vein, which we and other investigators (12, 13) used for application of the substances tested. Especially with regard to the components of the kallikrein-kinin system, these conditions may have led to a loss of the substances by inactivating enzymes or tubular secretion and subsequent excretion (5).

With regard to this possibility we investigated the vascular conditions in the catfish, which reacted essentially in the same manner as the shark. Figure 3a is a X-ray picture of an endoradiography of a part of the venous system immediately after injection of contrast medium, the volu-

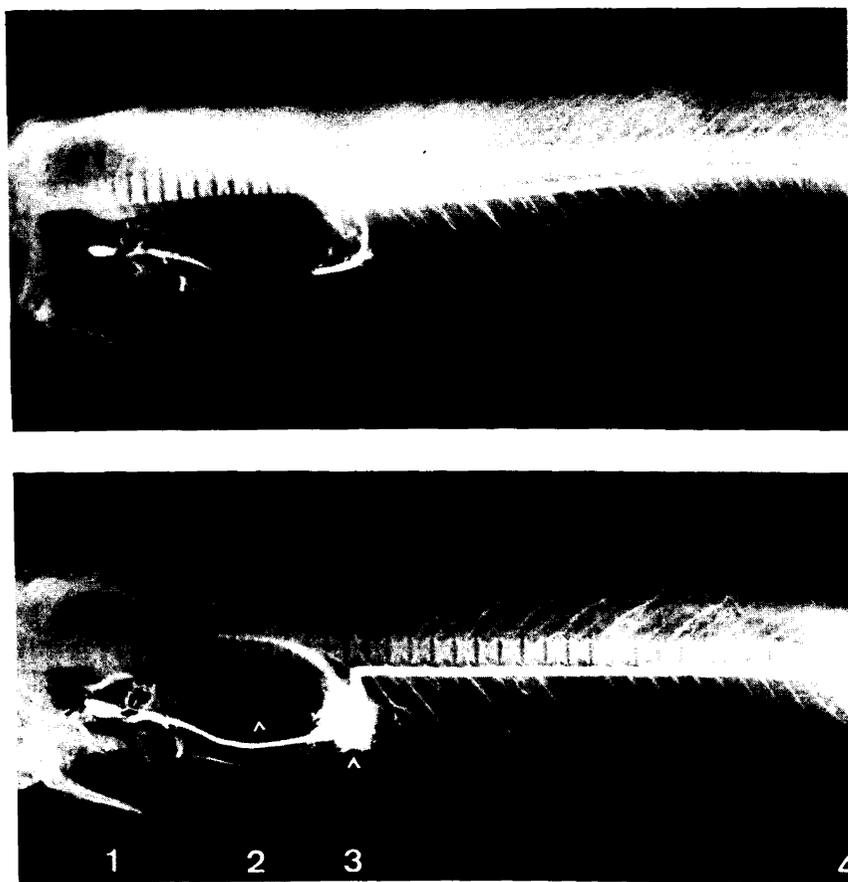


Fig. 3

X-ray picture of catfish venous system

- a) first injection of contrast medium (1.5 ml)  
 b) second injection of contrast medium (1.5 ml) 5 min later  
 1: heart  
 2: swimbladder  
 3: kidney  
 4: injection canula in the caudal vein

me of which corresponds to the usual applied volume. As can be seen, the contrast medium immediately advances to the heart. From figure 3b there can be seen the beginning of the distribution of the contrast medium into the capillary system of the kidneys. These findings are evidence for the fact that the passage of substances from the caudal vein to the common circulation is not hindered and that the circulation of the kidney is effected by a shunt. From the fact that all substances studied in part I and II of this paper were tested in both fish species with the same results, it can be concluded that the absent or slow reactions are real results.

In cartilaginous and teleost fishes there are no hints for

the existence of a kinin-liberating system comparable to that of mammals and birds. In spite of this there may occur *free* kinin-like peptides because in venoms of species of lower orders a number of preformed kinins were observed (for review see PISANO, 14). Perhaps there is no comparable regulating system in lower animals.

These investigations were partly performed at the "Naples Zoological Station", and we are very grateful to Prof. Dr. E. ROCCA and Dr. R. MARTIN for valuable advice.

We acknowledge financial support by the Deutsche Forschungsgemeinschaft.

We are indebted to Prof. Dr. M. PÖSCHL, Abt. f. Röntgenologie und Strahlentherapie der Chirurgischen Universitätsklinik München for the opportunity to perform the angiographies.

### References

- SCHIEVELBEIN, H., R. VOGEL and W. LORENZ, this Journal 7, 461 (1969).
- WEICHERT, G., Pflügers Arch. Physiol. 284, 147 (1965).
- MALVIN, R. L. and A. J. VANDER, Amer. J. Physiol. 213, 1582 (1967).
- WERLE, E., W. APPEL and E. HAPP, Naunyn-Schmiedeberg's Arch. exp. Pathol. Pharmacol. 234, 364 (1958).
- FREY, E. K., H. KRAUT, E. WERLE, R. VOGEL, G. ZICKGRAF and I. TRAUTSCHOLD, Das Kallikrein-Kinin-System und seine Inhibitoren. Enke-Verlag, Stuttgart (1968).
- VOGEL, R., Hoppe-Seyler's Z. physiol. Chem. 349, 926 (1968).
- WERLE, E., I. TRAUTSCHOLD, K. KRAMMER and A. SCHMAL, Hoppe-Seyler's Z. physiol. Chem. 349, 1441 (1968).
- ERDÖS, E. G., H. Y. T. YANG and I. MIWA. Paper read at 5. Konferenz der Gesellschaft für Biologische Chemie, München 1967.
- PRAHL, J. W., and H. NEURATH, Biochemistry USA 5, 2131 (1966).
- ZENDZIAN, E. N. and E. A. BARNARD, Arch. Biochem. Biophysics 122, 699 (1967).
- ROMER, A. S., The Vertebrate Body. W. B. Saunders Comp., Philadelphia-London (1966).
- LUTZ, B. R. and L. G. WYMAN, Biol. Bull. Lancaster 62, 17 (1932).
- SCHWARTZ, S. L. and J. F. BORZELLECA, Science Washington 163, 395 (1969).
- PISANO, J. J., Fed. Proc. 27, 58 (1968).

Dipl.-Chem. Dr. Rosmarie Vogel  
 8000 München 15  
 Nussbaumstr. 20