# Distribution of a Morphogenic Peptide Activating the Head Growth of Hydra in Rat Organs Following Intravascular Administration of $^3$H-Labeled Peptide

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The organ distribution of radioactivity following intravascular bolus injection of $^3$H-Lys-head growth activator in rats was studied. Two minutes after injection the renal level of radioactivity exceeded the blood level 7-fold; 80% of the total activity was bound with the blood cell membranes. An analysis of chemical derivatives of the labeled peptide in the plasma by means of reverse-phase high-performance liquid chromatography revealed the presence of several groups of radioactive metabolites with different hydrophilic properties. High-performance liquid chromatography of blood extracts obtained from samples taken 0.5, 1, 1.5, 2, 31, and 60 min after injection showed the transformation of initially hydrophobic head growth activator into more hydrophilic fragments. The $^3$H-Lys-head growth activator-associated radioactivity could be reliably detected in the blood only during the first two minutes after injection. The half-period of blood-to-organ distribution of $^3$H-labeled head growth activator lasted less than 30 seconds.

**Key Words:** *hydra head growth activator; tissue and organ distribution; pharmacokinetics; metabolism*

Hydra morphogenic peptide, or head growth activator (HGA), which governs head pole growth and tissue differentiation in the freshwater coelenterate *Hydra attenuata*, represents a undecapeptide pGlu-Pro-Pro-Gly-Gly-Ser-Lys-Val-Ile-Leu-Phe. It can also be isolated from the brain and intestine of mammals, including man. This is a unique example of a regulatory peptide which has preserved its structure during phylogenesis from the most primitive to the highly developed forms of life. Priority in the discovery of HGA in hydra, amino acid sequencing, and isolation from mammalian tissues belong to Bodenmuller and Schaller [7,14]. These investigators have also elaborated methods of HGA purification and quantitation in biological specimens [8,13]. The biological role of HGA in mammals including man is still little understood [1,4,5,10,11].

The goal of the present work was to study the distribution of $^3$H-Lys-HGA in rat tissues after intravascular bolus administration in order to estimate the rate of its clearance from the blood and to elucidate the pathways of its elimination.

## MATERIALS AND METHODS

The experiments were carried out on three male Wistar rats weighing 300-350 g. The anesthetized animals (pentobarbital sodium, 30 mg/kg) were administered via the femoral artery catheter 1 ml of

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$^3$H-Lys-HGA (63 Ci/mmol, 1 mCi/ml, diluted 1:4 with saline) in 30 seconds. Two minutes following the injection the animals were decapitated, and the organs were promptly removed and immediately frozen in liquid nitrogen, where they were further stored. The radioactivity was recorded in a Rack-beta scintillation counter (LKB) using a Brey scintillator (naphthalin, 60 g; PPO, 4 g; POPOP, 0.4 g; methanol, 100 ml; ethylene glycol, 20 ml; dioxane added to a final volume of 1 liter) that was added to the tissue samples solubilized in hyamine hydroxide (300 µl of hyamine hydroxide per 10-11 mg tissue). For a study of the kinetics of HGA in the blood, one anesthetized rat received $^3$H-Lys-HGA in the dose mentioned, and then, after washing of the catheter with saline, blood specimens were taken 0.5, 1, 1.5, 2, 31, and 60 min after injection and mixed with a double volume of Bennett's acidic solution [6]. The mixed samples underwent centrifugation, and supernatants were analyzed by means of high-performance liquid chromatography (HPLC). HGA adsorption on the blood cell membranes was evaluated by comparing the radioactivity of the total (untreated) blood with supernatant obtained after centrifugation of the blood lysed by freeze-thawing. For this, 400 µl of total lysed blood were centrifuged for 20 min at 3000 g. Supernatants were carefully poured off. Dense membrane sediment was mixed with 200 µl saline to a homogenous state with a glass rod, centrifuged for 30 min at 5000 g, and the supernatants were aspirated. Supernatants of both types were pooled and the radioactivity of the pooled supernatants was recorded using a Brey scintillator. In order to determine the applicability of Bennett's acidic solution for the extraction of HGA and its fragments from the blood and kidneys, the lysed blood was extracted with solution added at a 1:1 ratio (v/v); in the case of kidney tissue the ratio was 1:5 (w/v). Following the estimation of radioactivity the extracts were subjected to gradient reverse-phase HPLC (Si 100:Poly-ol:RP 18, 5 µ, Serva) followed by estimation of the radioactivity of fractions eluted per minute. Elution was performed with 10-40% acetonitrile gradient in the presence of 0.1% trifluoroacetic acid during 20 min with a flow rate of 1.4 ml/min. Under these conditions the retention time of HGA was equal to 16.5 min.

HGA was synthesized using the classical methods of liquid phase peptide chemistry. The HGA analog required for introduction of the $^3$H-label was obtained by the substitution of lysine residue for L-2,6-diamino-4-hexinic acid residue (Dah). As a result of [Dah7]-HGA catalysed hydrogenolysis, the final preparation, $^3$H-Lys-HGA with a specific activity of 63 Ci/mmol, was obtained [3].

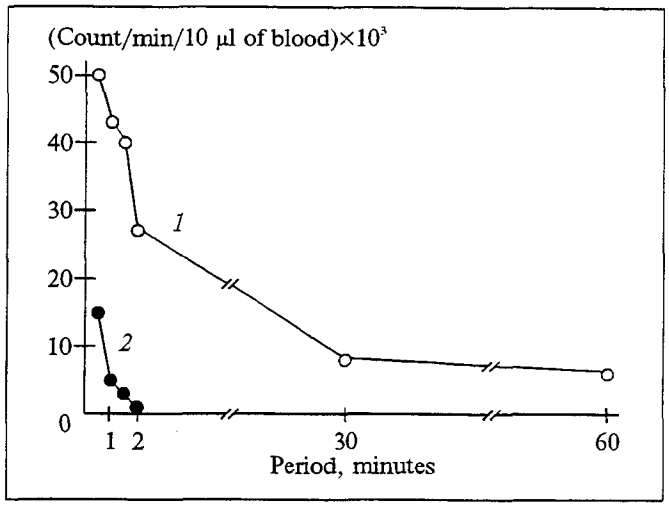


Fig. 1. Fall of the total radioactivity (*1*) and of the $^3$H–HGA (*2*) content in the blood in the first minutes after labeled peptide administration.

## RESULTS

The half-life of regulatory peptide distribution in the blood is known to proceed extremely quickly and lasts from a few seconds up to one minute [2,9,12]. Our data on the blood kinetics of $^3$H-Lys-HGA following intravascular bolus injection once more demonstrated this property of regulatory

TABLE 1. Distribution of Radioactivity among Rat Tissues 2 min Following $^3$H–Lys–HGA Intravenous Administration

| Organ (tissue) | Radioactivity, counts per min per mg tissue | Ratio: counts per min per mg tissue/counts per min per mg blood |
|---|---|---|
| Blood | 2833±674.1 | |
| Lungs | 1917±129.9 | 0.70±0.11 |
| Liver | 973±65.3 | 0.36±0.06 |
| Kidneys | 21048±6320.5 | 6.92±0.56 |
| Heart | 1217±128.75 | 0.44±0.06 |
| Spleen | 1197±127.3 | 0.45±0.09 |
| Thyroid | 2079±413.6 | 0.72±0.03 |
| Pancreas | 1659±236.9 | 0.63±0.16 |
| Stomach | 1372±298.5 | 0.47±0.02 |
| Adrenals | 1793±270.8 | 0.67±0.14 |
| Testes | 339±47.4 | 0.12±0.02 |
| Brain | | |
| stem | 242±0.03 | 0.09±0.03 |
| cerebellum | 208±55.9 | 0.07±0.01 |
| pituitary | 2596±338.6 | 0.93±0.10 |
| pineal body | 1840±266.6 | 0.73±0.25 |
| hypothalamus | 270±41.6 | 0.09±0.01 |

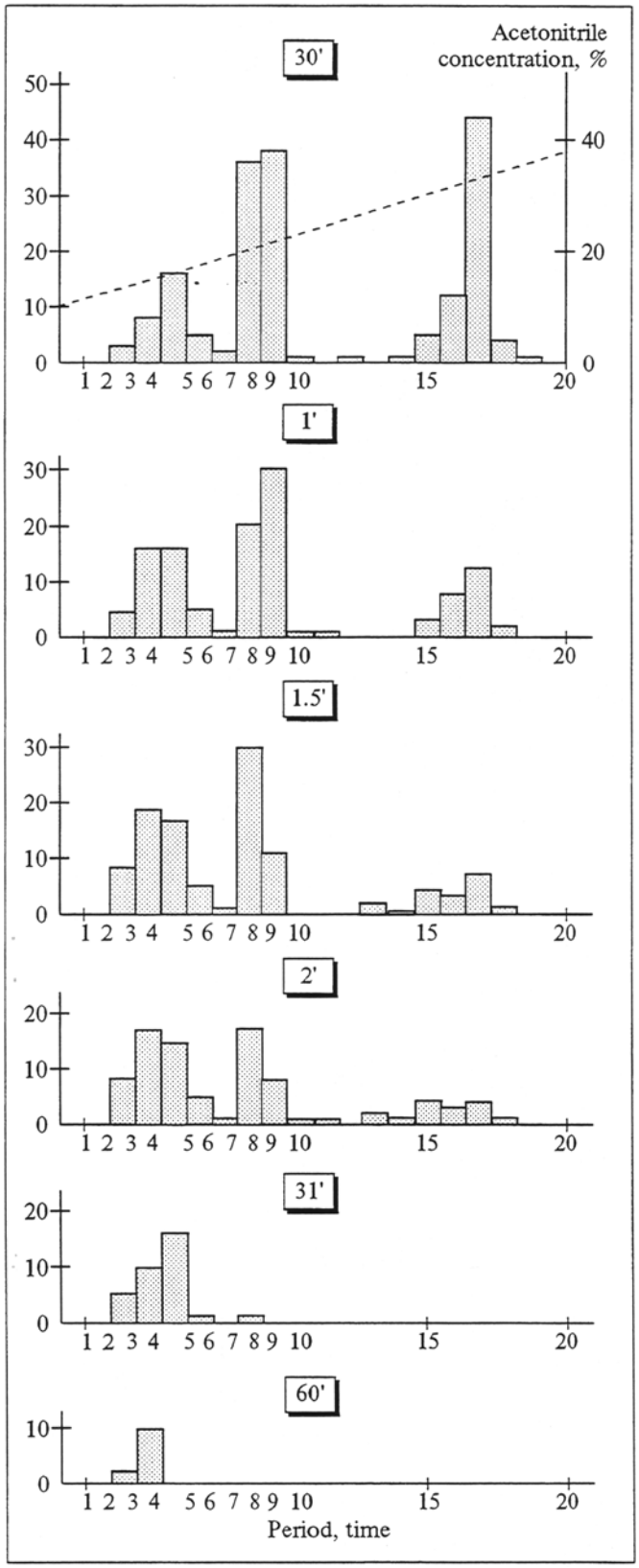


Fig. 2. Chromatographic division of blood extracts of one rat; blood samples were taken 0.5, 1, 1.5, 2, 31, and 60 min after $^3$H–HGA administration. Ordinate: radioactivity of per-minute elution fractions – (counts/min) $\times 10^3$. Dotted line reflects the elution profile.

peptides. As can be seen in Fig. 1, during the first two minutes after injection a drastic fall in both total blood radioactivity and blood HGA content is observed. Evidently, during these two minutes a redistribution of HGA from the blood into the tissues takes place. In fact, 2 min after $^3$H-Lys-HGA administration radioactivity is discovered in all the organs tested (Table 1). The levels of radioactivity in various tissues do not exceed the blood-associated radioactivity; the exception is the renal tissue, where the level of radioactivity exceeds that in the blood 7-fold.

HGA is strongly hydrophobic. This feature accounts for the high degree of its binding with the membranes of formed blood elements. In our experiment the binding of $^3$H-labeled compounds with the blood cell membranes of 3 rats two minutes after injection was equal to 76, 80, and 86%, respectively. The acidic solution extracted from the total blood 90, 89, and 79% of the radioactivity. Such a high efficacy of extraction provided the basis for using the solution in the pharmacological experiment mentioned above.

Chromatography of the acidic extracts (100 μl) of the blood samples taken during the first two minutes made it possible to follow the process of $^3$H-Lys-HGA elimination accompanied by the appearance of labeled fragments varying in hydrophobic properties (Fig. 2). *In vitro* experiments with human plasma [11] demonstrated the participation of angiotensin-converting enzyme in HGA metabolism. The half-life of the peptide in the plasma was 7 min. The authors suggested the participation of other enzymes, especially of tissue origin, in the biotransformation of HGA and its much more rapid degradation *in vivo*. Our results wholly confirmed this assumption. On the basis of the fact that 30 seconds after $^3$H-Lys-HGA administration in a dose of 2.5 μg (300 mln cpm) its blood concentration fell to approximately 12 ng/ml (1.4 mln cpm/ml), one can conclude that the half-life of the peptide in the blood is significantly less than 30 seconds and is hardly detectable in *in vivo* experiments.

The presented histograms reflecting the radioactivity of the per-minute elution fractions clearly demonstrate the time-associated transformation of the hydrophobic peptide into more and more hydrophilic fragments. This is shown by the appearance of 3 groups of radioactive metabolites that are eluted with 10-20%, 20-30%, and 30-40% acetonitrile solution, respectively. The highest rate of elimination is intrinsic to HGA itself and its radioactive fragments of similar hydrophobicity (fractions 16-17). Fractions 8-9 are eliminated more slowly. However, by the 31st min only the most

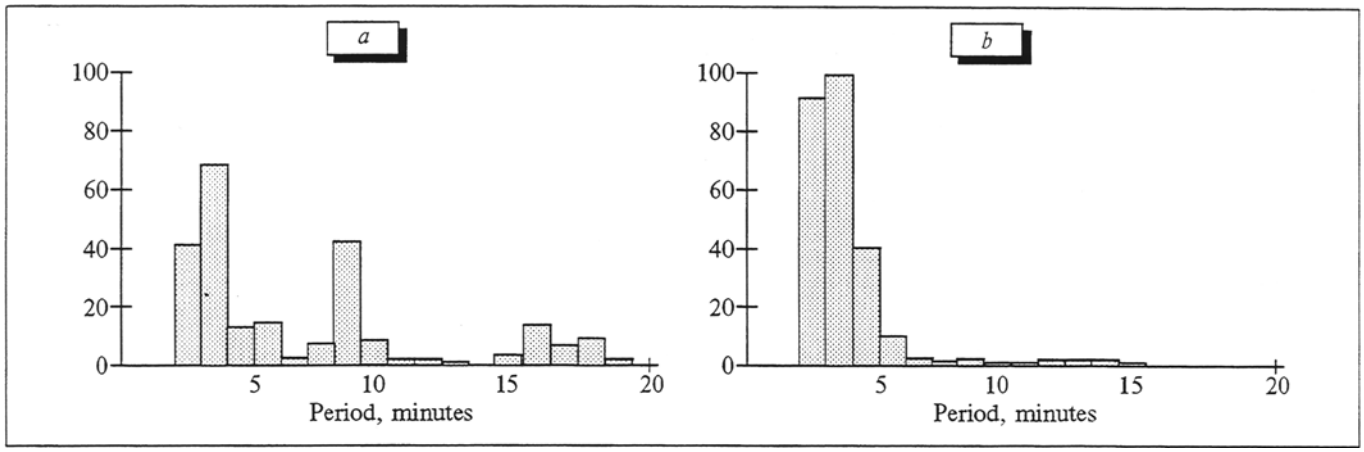


Fig. 3. Chromatographic division of blood (*a*) and kidney (*b*) extracts two minutes after $^3$H−HGA administration. Ordinate: radioactivity of per−minute elution fractions − (counts/min) ×10$^3$.

hydrophilic fragments that are eluted together with the front of the solvent (fractions 3-4) and immediately after it (fractions 5-6) can be detected in the blood. It is typical that the level of radioactivity of hydrophilic fractions 3-6 does not change during the first 2 min after HGA injection, slightly decreases toward the 31st minute, but remains rather high as long as 60 min following HGA injection. Apparently, just 2 min after injection the blood has no other $^3$H-metabolites of HGA, the further hydrolysis of which could maintain the level of the above-mentioned hydrophilic fragments; in addition, one should keep in mind that the radioactivity related to hydrophilic fragments is intensively cleared from the bloodstream by the kidneys. Therefore, the high level of HGA-derived hydrophilic fragments present in the blood 30 min or later following peptide administration is a result of transformation of the tissue-distributed peptide. As the lifetime of HGA in the blood is short, the main contributon to its metabolism is realized by the tissue enzymes.

The high hydrophobicity of HGA causes its immediate entrapment in tissues, despite the peptide binding to the membranes of blood elements. Possibly, it is the latter phenomenon that causes the broad spread of the peptide and its fragments within the organism. In view of all this, the 7-fold peptide concentration in the kidneys appears to be of particular interest. Unfortunately, attempts to extract the peptide or its derivatives from the renal tissue using Bennett's acidic solution resulted in obtaining as little as 40, 48, and 45% (data for 3 rats) of the radioactivity present in the kidneys. Chromatographic analysis revealed that all the radioactivity extracted was associated with most hydrophilic HGA metabolites (Fig. 3). Further investigations into the role of the kidney as an HGA clearing (or target?) organ would be of undoubtable interest.

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