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COMMENTARY

T-KININ AND T-KININOGEN—CHILDREN OF NEW TECHNOLOGY

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In the twenty-five years or so that I have been involved in the kallikrein-kinin field, nothing gave me more pleasure than being able to track down and purify a pharmacologically active peptide such as the leukokinins [1]. It never ceased to amaze me that a group of amino acids linked appropriately in peptide form could set off a chain of events leading to contraction or relaxation of a smooth muscle and that one could follow purification by the use of this bioassay. Even more startling is the fact that so few natural products can act as pharmacologically active materials. The act of bioassay was, and is a pharmacologist's province and provided for many years an expertise which kept kinins and related agents in the discipline of pharmacology but at the same time prevented others from being interested in kinin research. The complex enzymology and biochemistry involved did bring into the kinin investigative family a limited number of biochemists, organic chemists, and molecular biologists. In addition, since kinins are essentially pathological materials, an interested group of disease-oriented individuals added an important clinical dimension. There was considerable difficulty, however, in the ability of the kinin field to attract a broader group of investigators. The reason was the inability of investigators in other fields to carry out experiments in kinin research unless they were carefully tutored in the methodology and complexity of bioassay and its pitfalls in quantifying the minute quantities of kinins released by *in vivo* and *in vitro* reactions. The evolution of radioimmunoassay procedures altered this problem to a great extent. Investigators who learned radioimmunoassay as a general technique could now get involved with kinin assays in natural and pathological fluids more readily. One drawback to this evolution in technique, however, was that the specifics in terms of the kinin assayed were lost from sight because bradykinin, kallidin and Met-lys-bradykinin (the known releaseable kinins; Fig. 1) could not be differentiated from one another even by the most sophisticated of radioimmunoassay techniques [2]. Stewart and Morris [3] must be credited with initial attempts at advancing the state of the art in terms of identification by their initial publications on the use of high performance liquid chromatography (HPLC) to separate and identify kinins. T. K. Narayanan and I were stimulated by this research, and we decided on a long-range program to carefully study the ability of HPLC to separate the known kinins following *brief* purification procedures. In addition, we studied the use of fluorescamine-derivatized kinins to enhance column separation since our laboratory had enjoyed previous success separating these kinin derivatives by gel electrophoresis [4].

Following a year of such study, we reported at the International Kinin Congress in Munich [5] that such HPLC techniques were feasible and could provide in the future the chemical basis for detection and quantitation of all three known kinins. We have since published a full report on this topic [6]. The major point is that no longer do we have to guess at what kinin we have quantitated by radioimmunoassay but we can specifically identify the kinin by HPLC (Fig. 2). After we published the initial findings, Hiroshi Okamoto, in our laboratory, discovered that trypsin, in larger concentrations than generally employed, enhanced the release of kinin from rat plasma by 6-fold [7]. By usual methodology, the additional kinin would not be specifically identified. However, following purification procedures, Okamoto used our newly developed HPLC methodology employing direct and derivatized samples for chromatography. He discovered that the kinin released by trypsin did not elute at the time interval of any of the known kinins [8]. We decided to call the unknown kinin "T-kinin" (for trypsin-releasable kinin). Following purification of the peptide by the same HPLC procedures, it was sequenced and found to be Ile-Ser-bradykinin (Fig. 2) [8], a bradykinin homolog never before described. Within weeks after the discovery, John Stewart synthesized T-kinin, and we verified that the synthetic and the natural product were the same chemically and by pharmacological assay [9]. The concept of T-kinin was further supported by the amino acid analysis of a vasoactive peptide formed by an acid protease as described by Bedi *et al.* [10].

The discovery of T-kinin has several important meanings. First, it clearly shows that HPLC technology has changed much of our previous methodology. It is no longer satisfactory to assume that the kinin that one bioassays or quantitates by radioimmunoassay is bradykinin. HPLC clearly is an important method that one should and can use to identify the kinin components in physiological and pathological fluids. Used as we originally published [6] or in conjunction as we now also use with radioimmunoassay, it is a powerful and sensitive tool.

Another important meaning of the discovery of T-kinin is that our long held concepts that kininogens all contain the sequence Lys-bradykinin (either as LMW or HMW kininogens) are incorrect. The amino acid sequence of T-kinin does not contain lysine and

Bradykinin	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
Lys-bradykinin	Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
Met-Lys-bradykinin	Met-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
T-kinin	Ile-Ser-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg

Fig. 1. Structures of bradykinin and homologs.

clearly means that a third kininogen is present in rat plasma, i.e. T-kininogen. In fact, in normal rat plasma *T-kininogen is the most abundant kininogen* making up over 60% of the kininogen that can release kinin by trypsin. HMW kininogen holds only 10% of the releasable kinin while LMW kininogen holds 30% [11]. Of great interest are the recent findings by Barlas in our laboratory that rats injected with Freund's complete adjuvant (to produce arthritis) exhibit a huge increase in total plasma kininogen levels, with T-kininogen making up *over 90%* of the kininogens present [12]. This may clarify previous investigators' findings that noted this increase in total kininogen in adjuvant arthritis but could not by the techniques available realize the important role T-kininogen played in this increase.

Another very interesting and perhaps exciting prospect of the T-kinin discovery is that the T-kinin–T-kininogen system may have a completely different set of kinin-liberating enzymes that the kallikrein enzymes that liberate kinins from HMW and LMW kininogens. Neither kallikreins of tissues nor plasma seem to release T-kinin from its precursor in rat plasma. Also, unusually high concentrations of trypsin are required to liberate T-kinin when it is present in rat plasma. Is T-kininogen unusual in its structure to impair kinin release by naturally occurring enzymes but not by enzymes induced by pathological conditions? Does it circulate with a trypsin inhibitor? The recent isolation of T-kininogen from rat plasma by Okamoto should provide a number of answers to these questions.

How important will T-kinin and T-kininogen prove to be in human disease? It is important to survey plasma levels of human kininogens, especially in inflammatory diseased states, to determine if T-kininogen or other kininogens which differ from the known HMW and LMW kininogens may be detected. The new technology makes this possible.

My late and dear friend M. Rocha e Silva, the discoverer of bradykinin, always sought to educate people about the importance of kinins. Fortunately, a few months before his death he learned of our work from a visit I made to Brazil. He was delighted to learn about the new technology and that bradykinin was part of T-kinin. He encouraged me to write about it, and I wish to dedicate this review of T-kinin to him. We will miss his encouragement and enthusiasm.

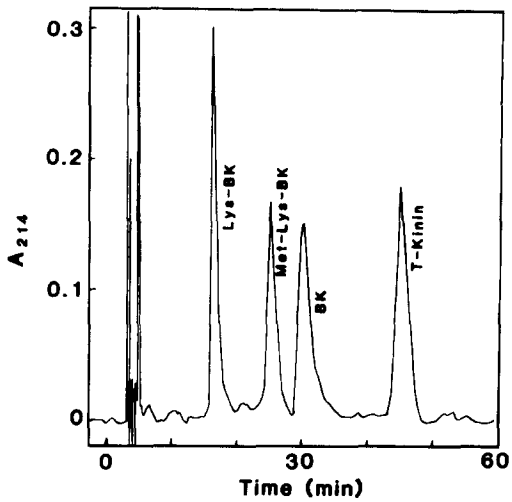

Fig. 2. Separation of kinins on a reverse-phase HPLC. Column, Ultrasphere ODS (5 μm), 25 × 0.46 cm. Eluent: 18% acetonitrile/82% 0.04 triethyl-ammonium formate, pH 4.0. Flow rate, 0.75 ml/min. A mixture of synthetic kinins and purified T-kinin from rat plasma (each 1 nmole) was injected at 0 min. Peptides were monitored by the absorbance at 214 nm.

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