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Current Concepts in Kinin Research

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1. Functions and Mechanisms of the Kinin System

Introduction

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Owing to the increasing general interest in the kallikrein-kinin system I can state with assurance, it is now slowly but surely coming into full focus. We know that it can no longer be regarded as an isolated system. The kinins, by interfering with angiotensin II formation and by activating phospholipase A_2, thus increasing prostaglandin formation as well as changing the ratio of prostaglandin E to F through the activation of 9-ketoreductase, go far beyond their original boundaries.

It is proper to recall history in a few lines.

It was Frey (1926) who found a blood pressure lowering substance in urine. This, he, Kraut and Werle later named kallikrein, because a similarly acting substance was found in the pancreas. Both of them are enzymes and they are closely related. It was Werle (1937) who proved that these enzymes do not act directly but that they release an effector substance from an inactive precursor in plasma. Werle (1948) named this effector substance kallidin. Much later it was shown by Werle, Trautschold and Leysath (1961) and by Pierce and Webster (1961) that kallidin is the lysyl derivative of bradykinin which was first described by Rocha e Silva, Beraldo and Rosenfeld (1949). By then the releasing enzymes, as well as the precursors and the effectors of the system had been described. After the initial discovery decades elapsed before the characterization of the kininases completed the system. It was Erdös and Sloane (1962) who described kininase I and then Erdös and Yang (1966, 1967) and Yang and Erdös (1967) who described kininase II. It was again Erdös and his co-workers who finally linked the kallikrein-kinin system to the renin-angiotensin system by proving the identity of kininase II with angiotensin I converting enzyme. Erdös and co-workers (1970, 1971, 1972) were able to demonstrate the conversion of angiotensin I by kininase II and they purified kininase II from lung and showed its identity with angiotensin I converting enzyme. Meng and Haberland (1973) discovered the effect of the kallikrein-kinin system on glucose uptake and metabolism; findings which lead nearer to the understanding of the basic functions of the system and which, at the same time, show how complicated the system is. In our field analytical procedures are very difficult, therefore clinical medical research became only slowly engaged. However, the chromogenic substrates developed by E. Amundsen and co-workers (1974) opened up a new and promising road here. By collecting piece after piece and, figuratively speaking, by trying to fit these bits of stone into a design in mosaic, I am sure that soon we shall get a clear insight into that part of physio-

logical chemistry which plays an important role in the regulation of the dynamics of a very complex system.

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Opening Remarks

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It was a great distinction for me to be asked by the Organizers of this Symposium, to give the Opening Remarks, to start such a fine gathering of workers in the field of Kinins. It is quite reasonable to detach a discussion on such a specialized field from the enormous mass of data that go through an International Pharmacological Congress, as the one we just attended. To attend the work presented in the Kinin field to the VII International Congress of Pharmacology, it would be necessary to use a computer to analyse the program, and even so, there would be neither time, nor physical possibility to know what are the last advances in the Kinin field. Furthermore, so many new facts are making their appearance at such large pharmacological Congresses, with about 3,000 communications and 5 or 6 thousand people attending the Congress, that going through so many papers to grasp what has been said about the Kinins, would be the same as tasting fine French wines, going from one counter to the other in small luncheon places, all over Paris. I consider that the right answer to such a massification of Pharmacology or any other branch of natural sciences, is to rely upon Satellite Symposia as the one we are attending here, and we are certainly grateful to Profs. Ulla Hamberg and G.L. Haberland who succeded in assembling us to-day, and also the attending audience of fine Kininologists, who prefered to come here, instead of profitting of such a beautiful Saturday in Paris.

We had many times the experience of choosing places for similar symposia, as the one we had in Rio, in December 1976, on the participation of "Kinins in the Inflammatory Reactions" published recently in Agents and Actions, vol. 8 nº 1/2 (1978), to which many of those present here came. It is always a dilemma, to choose a beautiful touristic place, such as Rio, and risk the attendance to the scientific meeting, in favor of the beaches and mountains, or to have the Symposium in an ugly place, and risk disinterest of the possibly attending scientists. It is one more reason to congratulate to organizers of this Symposium to risk the strong competition with Pompidou's Center, Tour Eiffel, Champs Elysées, for having so many people attending this excellent scientific meeting here at Rue de l'Observatoire, Paris 6eme.

It is difficult to choose items to present in a few short "Introductory Remarks", to be given in 15 minutes. Since we are going to discuss mainly the mechanism of release or formation of kinins, especially "Current Concepts in Kinin Research", I have to show in a few slides what are the basic data that have been established on the occurrence and release of bradykinin and related kinins (BK, Lys-Bk and Met-Lys-Bk, to mention only those that constituted basically the field of kinin research).

To give a succint idea about the field, I show the slides presented in Figs. 1 to 4, on the chemical composition of bradykinin and its release by the venom of Bothrops jararaca and by trypsin. Fig. 5 has been also presented many times and can be found in my book on Kinin Hormones (1970). Fig. 4 is presented to this Symposium in hommage to Ulla Hamberg, since in 1957, while in our laboratory in São Paulo, at the Instituto Biológico, for the first time we have shown that release of bradykinin by B. jararaca venom and by trypsin was dependent upon the specificity of proteases toward BAME, used as substrate (Hamberg, U. and Rocha e Silva, M., Experientia 13, 489, 1957). I think that Figures 1 and 4 are self-explanatory. They have appeared in other places, especially in the mentioned book on Kinin Hormones. Such demonstration of release of a biologically active peptide by partial hydrolysis became ever since a productive model to study other peptides and other substrates, as many papers in this Symposium will illustrate.

Figs. 6 and 7 give a clue to the work that is currently being done in our laboratory in collaboration with E. Santos and Hanna A. Rothschild "On the mechanism of activation of the pre-kininogenin-kininogenin (pre-kallikrein-kallikrein) system by sulfated polysaccharides and kaolin" and that will be presented as a short paper to this symposium.

As shown in Fig. 5, we had postulated, on the basis of activation of bradykinin by sulfated polysaccharides (heparin, cellulose sulfate, dextran sulfate) and sulfated polymers (such as polyethylene sulfate and others) that the activation process gears with the phase involving active products derived from Hageman Factor and blood clotting, to release plasma enzymes (kininogenin or plasma kallikrein) that will act upon BKg to release Bk. Therefore, we had postulated the important role to be played by natural sulfated polysaccharides to release bradykinin.

Later on, things took a different trend mainly in NIH and in San Diego (California) through the work of the group of Kaplan, Weiss, Wuepper and Cochrane who have shown that Hageman Factor, thromboplastin antecedent (PTA) identified with pre-kallikrein (or pre-kininogenin) itself through a feed-back loop would participate in the activation of the kinin system in plasma.

A simplified scheme of these processes is presented in Fig. 7, from a publication by Wuepper in the Proceedings of the Symposium held in Reston, Va., in 1974. Minor additions have recently been introduced in such a scheme, that would not alter substantially our point here.

The main point in the presentation of the results of our group, in Ribeirão Preto, is to raise the possibility to combine those schemes in a more complete one presented in Fig. 4 of the paper by Santos et al. (These Proceedings p.149.) By using the synthetic substrate Chromozym (Pentapharm, Basle) there was evidence that the pathways of activation by contact with kaolin which acts through its negatively charged surface, on one hand, and by addition of soluble sulfated polysaccharides (cellulose sulfate and heparin) on the other could be split in two (partially) distinct mechanisms, that funnel into a common mechanism. As far as the inhibitory effect of hexadimethrine bromide (Polybrene) is concerned, it acted much more powerfully upon activation by sulfated polysaccharides than upon activation by kaolin, but nevertheless it still acted, when present in sufficient amounts, upon the pathway initiated by contact with kaolin.

It is pertinent to remind you that in the Minireview published in 1974, in Life Sciences, vol. 15, pg. 7-22, on Present Trends of Kinin Research, we said textually: "... we still have an unknown pathway that might involve activation of a factor to start the whole series of reactions triggered by contact with

kaolin. In this connection we may call upon the phenomenon extensively studied in our laboratory of activation of the endogenous system with sulfated polysaccharides or sulfated polymers. Heparin is one of those substances that in proper concentration can activate the kinin system; cellulose sulfate and other sulfated polymers, such as polyethylene sulfate constitute a class of agents which are able to activate the kinin system in plasma. On the other hand the anti-heparin agents, protamine sulfate and hexadimethrine bromide (Polybrene) have largely been found to block the activation of the endogenous kinin system in plasma. Even the release of kinin under the conditions of "thermic edema" was found to be blocked by hexadimethrine bromide and more recently, Armstrong has shown that the effect of hexadimethrine bromide in preventing formation of Pain Producing Substance (a kinin from plasma) was due to interference on activation of the Hageman Factor. To tie up all the results presented above one might postulate the existence in plasma of a factor or pre-factor that might have the nature of a heparin-like or a sulfate-like material that could be able to play the role of kaolin or glass, and can be quantitatively removed by protamine sulfate or hexadimethrine bromide.

To end up with these Introductory Remarks I wish you a happy journey to compensate for the lost journey of strolling over Paris. Thank you.

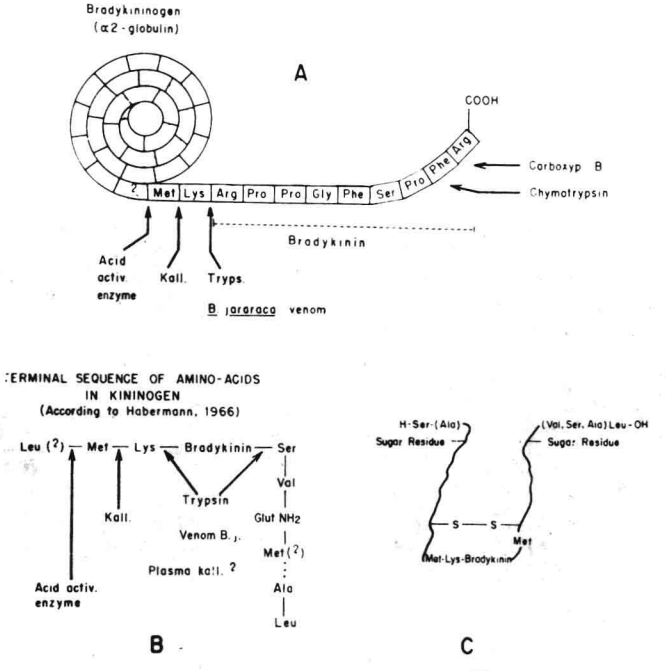

Fig. 1 - Pictorial view of the structure of bradykininogen with the attachment of the bradykinin molecule. A. Bradykininogen and related peptides at the end of the globulin molecule. B. Structure according to Habermann (1966). C. Gross structure of kininogen II according to Suzuki et al. (1967). From Rocha e Silva (1970).

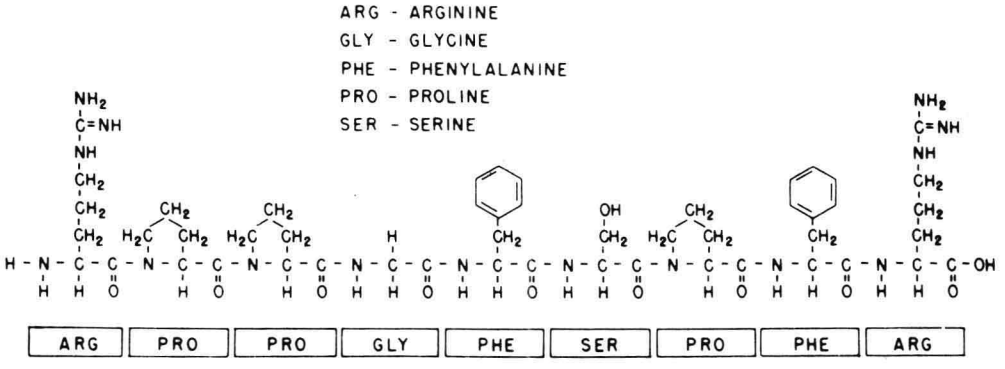

Fig. 2 - Formula of bradykinin

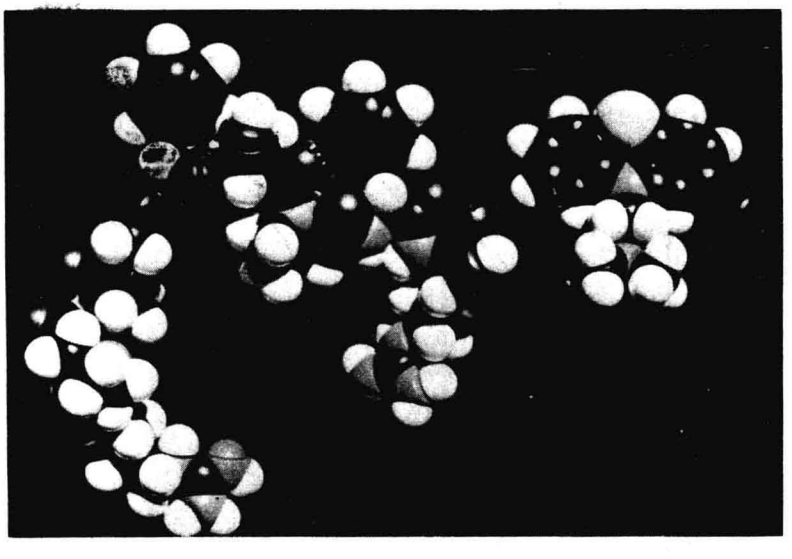

Fig. 3 - CPK model of the bradykinin molecule. In the right. The model of the molecule of chlorpromazine an antagonist competitor of bradykinin.

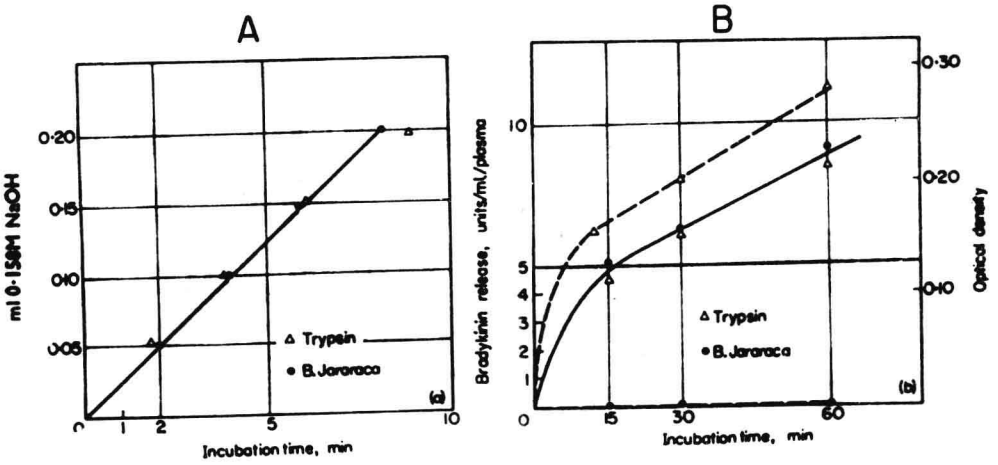

Fig. 4 - A. Equipotent solutions of trypsin and the heated B. jararaca venom using BAME as substrate. B. The same solutions were incubated with denatured plasma for the release of bradykinin (Δ) and the protein split products measured for trypsin (Δ) and the venom (·) after precipitation of the mixtures with TCA. With trypsin the whole substrate came into solution in TCA upper curve), though with the venom, practically no split product could be detected after one hour of incubation (lower curve —·—·—·—). (According to Hamberg and Rocha e Silva, 1957).