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COMMENTARY

THE KININS

A STATUS REPORT*

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Although kallikrein was discovered half a century ago, recent years have seen involvement of the kallikrein–kinin system in other areas of research, so much so that it is increasingly difficult for many to put this system into perspective. The purpose of this commentary is to give an overview of the field as related to other research areas.

When I started to think about writing a brief status report on kinin research, it did not occur to me that Professor Eugen Werle's death would make the need for such an article more obvious, since the development of this field cannot be separated from Werle's work. I am still fascinated to see that the factors he observed by doing simple experiments many years ago are still with us and gaining in importance. An appraisal of his many contributions, however, has to be done elsewhere.

The trend of the development in kinin research has been toward the complex. That is, the initially described simple reactions appeared to be more and more complicated later. We are slowly discovering that, in addition to their direct actions, the components of the kallikrein–kinin–kininase system play active roles in other systems such as the coagulation or complement system in blood, and that they can release or enhance the actions of other vasoactive and pain-producing substances.

I should like to describe briefly some recent findings that I consider interesting because either they explain the mechanism of some biological phenomena or they may indicate new developments. Just as the remarks of a bird watcher should not be mistaken for a handbook of ornithology, a kinin watcher does not necessarily write a comprehensive review. Thus, here it is, as I see it.

A complete description of all the interrelating metabolic cycles in blood that either result in the activation and inactivation of the plasma kallikrein–kinin system or are affected by plasma kallikrein could show more intersecting circles than the Olympic emblem. In addition to the names of various factors coined some time ago from Greek words, we may have to remember the names of various patients (e.g. Fletcher and Hageman) whose families, for reasons known only to Mother Nature and her geneticists, lacked one or another blood-borne factor.

A relatively simple scheme showing the processes which lead to activation of kallikrein and to the subsequent release of a kinin and its inactivation in blood is shown below (Fig. 1). The reactions and the components involved are numbered and discussed in sequence.

This scheme illustrates what happens in blood when plasma kallikrein is activated. The activation of tissue prekallikrein is either relatively simpler or we know less about it. Some recent findings dealing with plasma and tissue kallikrein systems are discussed together under the same heading below.

The basic reactions which lead to the release and inactivation of a kinin were described in 1937 by Werle *et al.* (Ref. 1; Fig. 2). As we understand this process now, plasma kallikrein is a zymogen which can be activated by Hageman factor or by its fragment. The active kallikrein cleaves kininogen and releases bradykinin (tissue kallikrein releases kallidin; Fig. 3). The free kinin is rapidly inactivated by kininases, but part of kallidin may be converted to bradykinin prior to its inactivation [2].

Prekallikrein and its activators (1,2,3). Hageman factor (Factor XII) has a molecular weight (m.w.) of about 90,000 [3, 4]. Contact with a negatively charged surface such as glass activates Hageman factor. The resulting active factor initiates blood clotting *in vitro* [3], and converts plasma prekallikrein to kallikrein [3, 4]. It can also activate a prekallikrein in the colon wall [5]. Active Hageman factor is converted to a fragment of about 35,000 m.w. which is less effective than the native enzyme in initiating the coagulation of blood, but is fully effective in the activation of prekallikrein [3, 4, 6]. Only a portion of Hageman factor present in plasma is activated by charged surfaces. The remainder can be converted enzymatically by plasma kallikrein [7] through a positive feedback. It was suggested [8] that plasma kallikrein cleaves Hageman factor in two places. If the m.w. of the factor is estimated to be 80,000, kallikrein would cleave it to fragments of 40,000, 52,000, 28,000 and 12,000 m.w. The 28,000 m.w. fragment is thought to be the prekallikrein activator [9]. This low m.w. derivarious active principles in blood which Webster diate, the plasminogen proactivator [7, 10]. Thus, the various active principles on blood which Webster [11] observed to be capable of activating prekallikrein are not separate enzymes but products of enzymatic conversion. In contrast to human blood, the Hageman factor of bovine blood is not converted to a lower m.w. derivative, but the whole molecule acts as prekallikrein activator [12].

These findings indicate that plasma kallikrein not only releases a kinin but also has other functions in plasma. For example, since Hageman factor can play a role in complement activation [13], plasma kallikrein may also participate in this reaction, or in the

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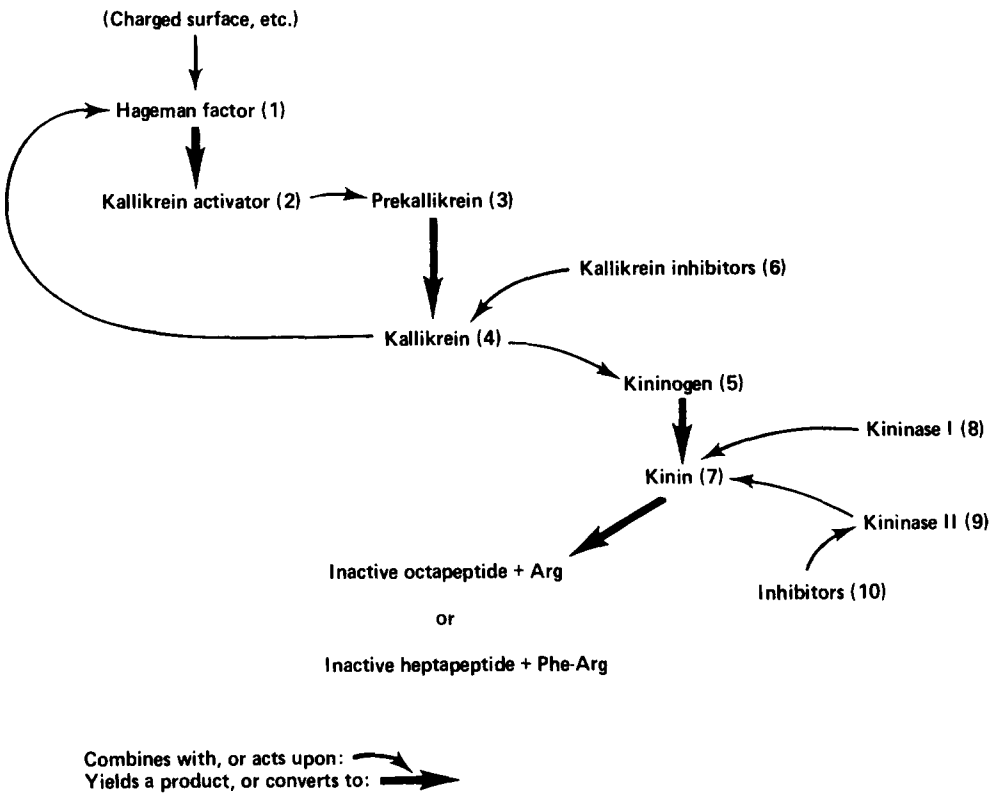

Fig. 1. The kallikrein–kinin–kininase system in blood.

activation of Factor VII in blood coagulation [14]. The idea that plasma kallikrein has additional roles in blood clotting is supported by the observation of a new familial syndrome, the Fletcher disease. This genetic defect results in prolonged clotting time *in vitro* of the blood of afflicted persons and can be corrected by adding a specific factor from normal plasma. The missing factor is identical with prekallikrein [15].

The conversion of Hageman factor to a lower m.w. derivative may also account for the factor Pf/Dil described as a mediator of vascular permeability. Activated Hageman factor or its fragments are probably identical with the permeability factor Pf/Dil that is released when normal plasma is diluted [7, 16, 17].

There are some conditions which are associated with the lowering of the prekallikrein level in plasma. Cirrhosis of the liver in alcoholics is one of these [18, 19].

Kallikrein (4, and tissue and urinary kallikreins; EC 3.4.21.8). Since the discovery of kallikrein, attempts have been made to connect the vasodilator effects of kallikrein with the regulation of blood pressure. Elliot and Nuzum [20] and later Werle and Korsten [21] observed a decreased excretion of urinary kallikrein in patients with essential hypertension. Recently, Margolius *et al.* [22–25] have investigated the role of urinary kallikrein in hypertension in patients and in rats by using radioactive [^{3}H]tosylylarginine methyl ester ([^{3}H]Tame) as a substrate for kallikrein. The [^{3}H]Tame assay is quite sensitive, although of limited specificity. It can be used to assay kallikrein if other proteases are not present in sufficient quantity to cleave the protected arginine ester.

Using radioactive and other substrates, investigators found lower than normal levels of kallikrein in urine of hypertensive patients and rats [22–27].

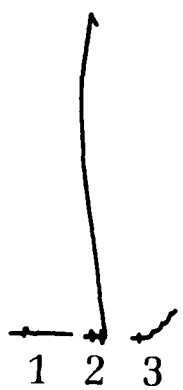

Fig. 2. Liberation of kallidin by kallikrein *in vitro*. Isolated surviving guinea pig colon. (1) Dog submaxillary gland kallikrein. (2) Kallikrein incubated with 0.8 ml human serum for 1 min. (3) Same as (2), but incubated for 5 min (see Ref. 1).

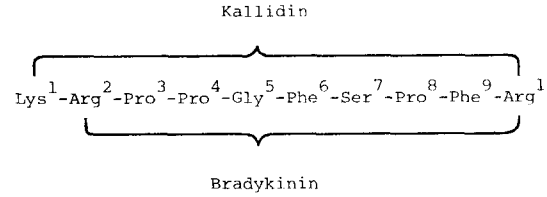

Fig. 3. Structure of bradykinin and kallidin.

Restriction of sodium intake or increased aldosterone level is associated with increased kallikrein excretion in normal humans [23]. Hypertensive patients excreted less enzyme under the same conditions [24]. In addition, vasodilators (PGE, acetycholine, isoproteranol and bradykinin) increase urinary kallikrein excretion [27a]. An additional factor which can affect kallikrein excretion is fluid intake because increased fluid intake is associated with increased kallikrein excretion [28–30]. It is likely that kallikrein in the urine is identical with kidney kallikrein [31, 32]. This is not as obvious as it first appears because the concentration and the demonstrated rate of biosynthesis of kallikrein in kidney [33] are too low to account for the high rate of excretion in animals such as the rat.

The physiologic significance of these changes in urinary kallikrein excretion as induced by changes in renal function and/or hypertension has yet to be proven. It is likely that the biological effects of renal kallikrein are brought about by enzymic release of kinins. Urine contains bradykinin, kallidin and met-lys-bradykinin [34], and at least three different kininases are present in the renal cortex [35]. Of the urinary peptides, met-lys-bradykinin is more resistant to kininase II [36] than the two other kinins. Kinins are natriuretic and increase renal cortical blood flow [37, 38]. The increased flow could be due to a direct and/or an indirect action. The latter is probably mediated by the release of E type prostaglandin (PGE) from the interstitial cells of the medulla [39].

Renal kallikrein is an enzyme bound to membranes. At first it was thought to be a lysosomal enzyme [40], but later it was detected in the microsomal fraction of homogenized kidneys [31, 32]. Recently a plasma membrane fraction was isolated from rat kidney which was rich in kallikrein [41]. The kallikrein-containing membrane can be separated from the brush border, indicating that kallikrein is not on the lumen side of the proximal tubular epithelial cells [41]. Kallikrein in urine may originate from the distal tubules [41, 42].

Kallikrein is present in some white blood cells. Polymorphonuclear leucocytes contain a kallikrein that releases a kinin at a neutral pH [43–45, 45a].* Furthermore, at an acid pH, polymorphonuclear leucocytes and macrophages release leukokinin, which is different from bradykinin as it contains 21 amino acids [46–48]. Leukokinin may be involved in generating ascites in murine neoplasia. Pepstatin, an acid protease inhibitor, inhibits the kininogenase that releases leukokinin and blocks the accumulation of ascites fluid [48].

Kininogen (5). Genetic studies have helped to solve another problem. The question of how many forms of kininogen, the substrate of kallikrein, exist in blood has been debated for many years. In 1966, the separation of two kininogens from a plasma globulin was reported [49–51]. One, the so-called high (100,000–200,000) m.w. kininogen [52, 53], appears to be the preferred substrate of plasma kallikrein, while the low (50,000–70,000) m.w. kininogen [54] is a substrate of glandular (tissue) kallikrein. Some investigators could confirm the data and purify high m.w. kininogen; others found only one low m.w. kininogen after purification of plasma kininogen [53]). Recently, several families (e.g. Fitzgerald, Williams and Flaujeac) have been found with a blood clotting deficiency *in vitro*. Since this deficiency could be corrected by adding purified high m.w. kininogen to the blood of the afflicted persons, this protein must have a function in blood clotting, in addition to being a substrate of kallikrein [55–57a]. This observation also may answer a somewhat philosophical question of why more than 98 per cent of the kininogen protein, the portion which does not contain the kinin sequence, would be "wasted"? Obviously, the whole kininogen molecule has a role in blood clotting.

Measurement of kininogen level has been used as an indicator of kinin release [58]. In young victims of trauma injuries complicated by sepsis, there was a good correlation between kininogen level and the outcome of shock. A drop in the kininogen substrate of plasma kallikrein to zero or near zero usually indicated a lethal outcome of the shock in hospitalized patients. The kininogen levels rose toward normal in those shock patients who survived [59]. Injection of live *Escherichia coli* bacteria also depleted the kininogen level to nearly zero in unanesthetized baboons [60]. Interestingly, infusion of epinephrine and norepinephrine into rats lowered the kininogen level in plasma [61].

In those species where kinin is located in the kininogen molecule in a position other than the C- or N-terminal end, kallikrein must liberate another peptide before releasing the kinin. One such peptide consisting of 41 amino acids and liberated from plasma kininogen was characterized by Han *et al.* [62]. This peptide contains a number of basic amino acids which suggests a biological activity such as histamine release, since other basic peptides release histamine [63, 64].

Kallikrein inhibitor (6). Some of the complications in kinin research have come from the ubiquitous presence of kallikrein inhibitors in blood and tissues [65]. Aprotinin (Trasylol), a peptide extracted from bovine lung, is identical with the pancreatic trypsin inhibitor of Kunitz. It has been widely used clinically outside of the United States. Among the many published reports, a recent double blind study [66] evaluated its efficacy in acute pancreatitis. The investigations of Trapnell indicated that aprotinin in large doses (4,200,000 units i.v.) was beneficial in the treatment of pancreatitis.

Before the dawn of affinity chromatography, kallikrein coupled to insoluble carrier was used for the purification of inhibitors [67]. Vice versa, aprotinin linked to a water-soluble matrix was employed for the purification of kallikrein [68, 69]. As a result, a variety of inhibitors and kallikreins were purified.

Kinins (7). I have not noticed the discovery of any significant new direct action of kinins. However, some recently described indirect actions of the peptides are worth mentioning. The venom of the wasp *Polistes* contains a kinin that consists of 18 amino acids [70]. The C-terminal half of the peptide (nine amino acids) is identical with bradykinin, but the N-terminal portion is different as it contains four basic amino acids.

* Human leukocytes from allergic persons released a kallikrein-like enzyme when challenged with antigen [45a]. The presence of calcium was essential for the release of the enzyme.

The peptide is an efficient histamine releaser, and its potency on rat mast cells approaches that of the synthetic histamine releaser compound 48/80 [63]. On the other hand, bradykinin and kallidin themselves are only weak histamine releasers [63, 71, 72].

The interrelationship of kinins with prostaglandins is interesting. As previously mentioned, kinins release PGE in the kidney [39], and that may be an important antihypertensive function [73]. Furthermore, bradykinin may be a mediator in inflammation also through the release of a prostaglandin. Prostaglandin may increase the algesic and edema-forming effects of the peptide. Thus, just as 5-hydroxytryptamine sensitizes tissues to pain caused by bradykinin [74], PGE enhances the effect of bradykinin on pain receptors and on capillary permeability [75, 76]. Whether these findings can explain some of the actions of nonsteroidal anti-inflammatory agents which suppress postaglandin synthesis remains to be seen.

Another recent observation may explain the paradoxical effect of bradykinin on blood vessels. Although bradykinin dilates arterioles, it constricts venules as shown by Guth *et al.* [77]. It was suggested that bradykinin releases the vasodilator PGE from the arteries, but it liberates the vasoconstrictor PGF from the veins. This finding may explain why bradykinin constricts veins and dilates arteries, provided the phenomena can be reproduced at a much lower concentration of bradykinin than used in the initial experiments (50 μg/ml; Ref. 78).

An additional important action of bradykinin was attributed to prostaglandin release. It has been known since 1932 that kallikrein is a potent coronary vasodilator, presumably through the release of a kinin. This was confirmed by using synthetic bradykinin [79]. The vasodilator effect of bradykinin in the isolated perfused rabbit heart can be blocked with indomethacin, indicating that it may be mediated through the release of PGE [80].

Kinins can increase the concentrations of cyclic AMP and GMP in lung slices. But, here again the concentrations of bradykinin required to induce transient accumulation of cyclic nucleotides are much higher (10–20 μg/ml) than those found in mammalian blood. Interestingly, the effect of bradykinin on cyclic AMP could be prevented completely by indomethacin, but this treatment did not change the accumulation of cyclic GMP in the same tissue. Thus, the effect on cyclic AMP may be mediated by enhanced prostaglandin synthesis [81].

Kinins are released from all mammalian plasma tested [2]. Alligator and turtle plasma contain a kinin system similar to the mammalian one. A kinin can be liberated in avian plasma, provided the prekallikrein activator is transferred from turtle or mammalian plasma. Other species such as amphibia, snakes or fish do not have the kallikrein–kinin system [82]. Pancreatic kallikrein added to fish or amphibian plasma [83] liberated an oxytocic agent that is probably not a kinin.†

There are a number of diseases where the amounts of free kinins increase in blood or exudates [79]. Recently, the appearance of free kinins has been emphasized in the postgastrectomy dumping syndrome, in certain transfusion reactions and in inflammatory joint diseases [84].

Kininases (8,9) and their inhibitors (10). Blood and tissues contain enzymes, collectively called kininases, which rapidly inactivate kinins. Several kininases are known, but the importance of some of them still eludes us. Cleavage of any one of the eight peptide bonds in bradykinin completely inactivates the peptide. Kallidin can be converted to bradykinin by an aminopeptidase that hydrolyzes the Lys^1—Arg^2 bond of this decapeptide. Although the enzyme was already discovered in 1962 [85], it was purified from liver only later [86] and recently from plasma [87]. In spite of the presence of this enzyme in blood and tissues, probably only a fraction of the liberated kallidin is converted to bradykinin at the N-terminal end before it is inactivated at the C-terminal end. Brain also contains kininases. One of them was recently purified from rabbit brain; it cleaves bradykinin at the Phe^5–Ser^6 bond [88].

The most important kininases have been named kininase I and II [85]. Kininase I is a carboxypeptidase called carboxypeptidase N or arginine carboxypeptidase (EC 3.4.12.7). Sometimes it is referred to as anaphylatoxin inactivator and has been described as "carboxypeptidase B-like enzyme in blood". Since pancreatic carboxypeptidase B of animal [89] and man* has different properties and a molecular weight of 34,000 (which would exclude any long sojourn in blood), the latter name is erroneous. From studies done with immunological techniques, it can be estimated that less than 0.1 per cent of the carboxypeptidase activity of human plasma may be attributed to pancreatic carboxypeptidase B [90]. Carboxypeptidase N has a molecular weight of 280,000 and contains subunits of 90,000 and 45,000 m.w. On standing, it easily dissociates to subunits which retain some enzymic activity, although they are less stable than the native enzyme [91, 92]. Carboxypeptidase N generally cleaves C-terminal lysine faster than arginine, except when alanine precedes the C-terminal arginine. An active peptide which is released when the complement system is activated, the C3a anaphylatoxin has a C-terminal Ala–Arg sequence [93, 94]. It was shown that C3a is readily hydrolyzed by carboxypeptidase N [91–93]. A number of peptides such as the nerve growth factor [95] and the epidermal growth factor [96] have a C-terminal arginine; thus, if they enter the circulating blood, they might be cleaved by carboxypeptidase N. Carboxypeptidase N probably originates from the liver [91, 92], but can be detected in other organs such as the lung [97] and possibly in the skin [98].

Streeten *et al.* [99] described a familial syndrome associated with orthostatic fall in pulse pressure, rise in heart rate, lightheadedness, etc. They attributed these symptoms to an abnormally low plasma kininase I activity.

More attention has been paid recently to the "newer" kininase, kininase II. This interest is due in

† The very extensive work on non-mammalian kinins was described in a review article by G. Bertaccini (*Pharmac. Rev.*, in press). He lists, for example, 12 different bradykinins. Extracts of venom sacks contain a heptadecapeptide and a pentadecapeptide, vespulakinin 1 and 2. In addition to the complete sequence of bradykinin these peptides have basic amino acids and carbohydrate components [83a].

part to the fact that the enzyme also converts angiotensin I to angiotensin II and that specific inhibitors are available to explore its functions *in vitro* and *in vivo* (for reference see [100, 101]). The enzyme is a peptidyldipeptide hydrolase (EC 3.4.15.1) which cleaves Phe^8–Arg^9 from bradykinin and His^9–Leu^{10} from angiotensin I. by this action it either inactivates the hypotensive kinins or activates the hypertensive angiotensin. Kininase II was concentrated first from a particulate fraction of the homogenized hog kidney and from human blood. It was observed later that this kininase is identical with the converting enzyme [35, 85, 100]. The enzyme is present in many tissues and cell types in the body. Most organs inactivate bradykinin rapidly, but the importance of the pulmonary circulation in the conversion of angiotensin I [102] and the inactivation of bradykinin [103, 104] has been especially emphasized. The vascular endothelium of the lung is a rich source of the membrane-bound enzyme [105]. Electron microscopy combined with labeled antibody technique localized the enzyme on the surface of the endothelial cells [106]. In addition, it is present in the kidney, in tubular epithelial cells including the brush border of the proximal tubules. This was shown *in vitro* by using immunofluorescent antibodies to converting enzyme [106a]. Brush border membranes separated from homogenized rat kidney by differential centrifugation [41] contained converting enzyme. The enzyme occurs also in high concentration in such diverse tissues as the testicles [107] and the choroid plexus [108].

Although kininase II cleaves both angiotensin I and bradykinin, bradykinin is the preferred substrate owing to its lower K_m. The larger kinin analogues such as met-lys-bradykinin are cleaved more slowly by the enzyme [36]. This fact may have some bearing on the functions of kinins.

Many compounds prolong the actions of kinins *in vivo*; most of these agents bind the metal cofactor of a kininase [35, 85]. Lately peptides have been synthesized that inhibit kininase II competitively [100]. Originally this type of inhibitors was found when snake venom was used to potentiate the action of bradykinin on the isolated guinea pig ileum [109]. Several peptides were purified from snake venoms and were later synthesized. They inhibit the inactivation of bradykinin by kininase II, and the conversion of angiotensin I to angiotensin II by the same enzyme. The nonapeptide SQ 20881 (BPF9a) has been used most frequently [101]. It blocks kininase II at a very low concentration (10^{-7} 10^{-8} M) but does not inhibit kininase I. This inhibitor has been employed to investigate the identity of kininase II and the angiotensin I converting enzyme, to block the conversion of angiotensin I during the perfusion of various organs or after i.v. injection into animals, and to determine the importance of renin in shock and in regulating blood flow in organs, etc. [38, 101, 110, 111]. Lately SQ 20881 (BPF9a) has been tested as an aid for diagnosing renin-related hypertension [112].

The concentration of kininase II in blood varies from species to species. For example, guinea pig plasma has a high enzymic activity but dog plasma has only traces of it [100]. Since many of the inhibition studies have been carried out in dogs, in these animals the inhibitor may block the membrane-bound tissue enzyme. In other laboratory animals, the inhibition of the blood-borne kininase II can also be important.

Kininase II [85] cleaves a variety of peptides other than kinins or angiotensin I [100]. Some of these are short synthetic peptides used as substrates in the spectrophotometer, fluorometer, or in the amino acid analyzer. Since the enzyme is inhibited by metal-binding agents, it must have a bivalent metal cofactor. Chloride ion activates the enzyme probably by inducing changes in the configuration of the protein molecule [91]. Kininase II has a lower m.w. than kininase I [85, 91, 101]. The lowest estimated m.w. of the rabbit lung enzyme is 129,000 [101, 113].

It seems that kininase I functions mostly in blood, while in tissues kininase II terminates the actions of kinins.

This brief survey of the literature indicates that the components of the kallikrein–kinin–kininase systems have functions other than the release and inactivation of kinins. They contribute to blood clotting, to the inactivation of anaphylatoxins, to the activation of plasminogen and angiotensin I, etc. Kinins themselves are hypotensive; they increase capillary permeability, cause pain and are chemotactic to leucocytes [2, 79], but in addition they release other agents such as catecholamines, histamine or prostaglandins, and can accentuate or modify the primary actions of the agents.

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