A New Biologically Active Peptide System in Serum Related to Classical Anaphylatoxin[1]

Classical anaphylatoxin, which causes histamine liberation and lethal anaphylatoxin shock in the guinea-pig[2], is formed in vitro in mammalian sera by contact reaction with various hydrophilic, insoluble substances of high molecular weight, e.g. dextran, yeast, bacterial endotoxic lipopolysaccharides or antigen – antibody complexes[2-4]. Formation of anaphylatoxin in vivo has been observed[5]. Beside histamine liberation, some other biological activities of anaphylatoxin are known, for example contraction of smooth muscle[2], coronary constriction[6], bronchospasm[2,3,7], enhancement of capillary permeability[2,4,8], and chemotactic activity for neutrophil leucocytes[9-12].

Different anaphylatoxins were reported to exist[13] which are generated from complement components C3 and C5 by immune and non-immune processes (e.g. protease[5,11] or venom action[5]). Although the origin of classical anaphylatoxin is definitely unknown, it may possibly be identical with anaphylatoxin generated from the complement component C5[5,14]. The biological importance of anaphylatoxins and their role in immune and non-immune processes has not been clarified. Their participation in any anaphylactic reaction remains to be demonstrated[2,3,15]. Otherwise, chemotaxis is thought to be an important trapping mechanism for the accumulation of different cell types on the reaction site of inflammation (for review see[16]). Therefore, the reported chemotactic activity of anaphylatoxins[9-12] and the enhancement of capillary permeability[2,4,8] might be effects of physiological significance, whereas the other known activities are probably more important for pathological reactions.

This report presents evidence for a new peptide system in mammalian serum, formed by immune and non-immune processes in vitro which is responsible for leucotactic activity and for the induction of different shock types. The hitherto unrecognized peptide system, with classical anaphylatoxin as peptide component, probably plays a key role in the mobilization of cellular defense mechanisms in vivo, and, in certain cases, is perhaps responsible for the pathogenesis of allergic diseases and of different shock types.

Methods. Details for the separation and crystallization of peptides and proteins and biological assays are described elsewhere[17-22]. The chemotactic activity was determined in vitro in the Boyden-chamber[23], as reviewed by Sorkin et al.[16]. For experiments in vivo, guinea-pigs of both sexes were used. Injection occured into the jugular vein under local anesthesia.

Results and discussion. The results show that confusion about anaphylatoxin activities, especially chemotactic attraction of leucocytes, is due to detectable impurities in anaphylatoxin preparations[17,18,24,25]: Two basic peptides have been isolated from hog, rat and guinea-pig serum after contact reaction of the serum with dextran, yeast or immune complexes[17,24-27]. They were crystallized in a molecular homogenous state[17,24-27]. One of the peptides, the classical anaphylatoxin(I), is the well-known histamine liberating principle in guinea-pigs. The molecular weight of the anaphylatoxins, isolated from hog and rat serum, is 9500, and from guinea-pig serum is 15,000, as determined by gel chromatography[17,24-27]. The second, newly detected peptide (II), called cocytotaxin[17,19,20,25], has a molecular weight of 8,500 (as determined by gel chromatography), and is also formed by the contact reaction, leading to the anaphylatoxin molecule[17,18]. The physicochemical behaviour of this basic peptide (II) is very similar to anaphylatoxin, but they differ in their biological activities[17,18,25]. While anaphylatoxin causes histamine liberation in guinea-pigs in vivo, leading to lethal anaphylatoxin shock under formation of lung emphysema, contraction of smooth muscle, coronary constriction, bronchospasm, and enhancement of capillary permeability (assayed on guinea-pigs), the crystallized cocytotaxin elicits none of these effects. Otherwise, neither homogenous anaphylatoxin, nor cocytotaxin show chemotactic activity for neutrophils as separated components, as determined in vitro in the Boyden-chamber[23]. However, recombination of the 2 peptides in a molar ratio 1:0.01 till 1:8 (I:II)[19,20-22] leads to chemotactic activity for neutrophils such as was ascribed earlier by many auth ors to anaphylatoxin preparations[9-12]. The chemotactic activity of this peptide system is not a linear function of total peptide concentrations, but it is an asymmetric sigmoid function, showing an activity maximum[20,21]. Hence, at certain molar ratios of the 2 peptides, no responsiveness of neutrophils to the peptide system in terms of chemotaxis is observed in vitro[20,21].

As leucotactic activity is dependent on the subtle interplay of the 2 peptides, anaphylatoxin activity of anaphylatoxin (I) is enhanced by cocytotaxin (II).

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Nevertheless, both, the leucotactic and the anaphylatoxin activity, are influenced in a different manner by the concentrations of the 2 peptides[21,22]. Therefore, leucotactic and anaphylatoxin effects represent different activity phases with only a partial overlap in certain concentration areas of the binary peptide system [21,22]. Thus, the homologous anaphylatoxin can act biologically (in terms of chemotaxis) as a component of the peptide system without displaying toxic effects itself (in terms of induction of lethal shock in vivo, and of guinea-pig ileum contraction in vitro). The evaluation of the existence of such partially overlapping activity phases of anaphylatoxin effects may explain physiological (e.g. mobilization of cellular defense mechanisms) and pathological reactions (e.g. some types of anaphylactic reactions) in which participation of anaphylatoxin in vivo is discussed (for review see[3,28]).

The anaphylatoxin (I) and the cocytotaxin (II) levels can be regulated by the activity of a leucotactic peptide system regulator enzyme (LSRE)[17]. This hitherto unknown enzyme, which has been highly purified[29], is most likely a peptidyl transferase or a ligase. The mechanism of action of this enzyme is under study, but it is conceivable that it catalyses a normal transpeptidation reaction as known from model studies[30,31]. In serum (hog, rat and guinea-pig), this enzyme is normally inactive, but it is activated by various particles with high surface activity[17] at neutral pH (e.g. certain lots of Sephadex G 25, certain types of chorcoal[29]). Active LSRE links the 2 peptides of the leucotactic peptide system, thus forming multiple proteins (refered as protein A, B, and C) with a molecular weight of 28,000, 56,000, and 112,000, respectively, as determined by gel chromatography[29]. The first hitherto identifiable product of enzymatic catalysis is protein A.

The isolated 3 proteins as metabolic products of anaphylatoxin and cocytotaxin show no chemotactic activity for neutrophils, neither alone nor in combination with anaphylatoxin or cocytotaxin[32]. In view of the fact that these proteins also contain the anaphylatoxin moiety, they were assayed for anaphylatoxin-like activities[32]: Protein A (mol. wt. 28,000) has similar activities to anaphylatoxin peptide (I). Shock induced by anaphylatoxin on guinea-pigs results in death after 4–5 min with histamine liberation and lung emphysema (preventable by antihistamines), whereas protein A causes a fatal shock with death after 5–8 min without formation of lung emphysema. Nevertheless, the behaviour of the animal resembles the behaviour during anaphylatoxin shock. Typical symptoms are dyspnoea and spasms. Protein B and C cause protracted lethal shock with death after 40–60 min or 2–12 h, respectively. Typical symptoms of this protracted shock are successive and alternating appearance of dyspnoea, excitation, sleep and drowsiness and, depending on the applied concentration of proteins, screaming spasms. Death is caused by circulatory insufficiency with symptoms of right ventricle dilatation and edema (especially after application of protein B, mol. wt. 56,000). Shocks induced by protein A, B or C cannot be prevented by antihistamine or protease inhibitors. The physiological function of these proteins is at present unclear, but obviously, they produce shocks which are similar to those protracted shocks observed in experimental anaphylaxis[3,33,34].

With the isolation of different mediators of shock on guinea-pig, many reported contradictory results on the biological action of anaphylatoxin preparations which have been produced by different contact substances (for review see[2,3]), might be explained by the interplay of different mediators, formed as a consequense of enzymatic reaction with anaphylatoxin as one of the substrates. Evidence remains to be established as to how far the isolated components of this system are activity principles in similar phenomena in vivo, such as the Arthus reaction for which the participation of anaphylatoxin is discussed[3,28], and the Shwartzman phenomenon[35]. Otherwise, on the basis of the biological activities of the reported components of the leucotactic peptide system, a hitherto unrecognized mediatory relationship is suggested between chronic inflammation, anaphylatoxic and other pathological reactions with symptoms of circulatory insufficiency and cardiac disease[36,37].

Zusammenfassung. Produkte einer enzymatischen Reaktion mit klassischem Anaphylatoxin als Substrat, das als Peptidkomponente des leukotaktischen Systems identifiziert und kristallisiert wurde, können als Mediatoren verschiedene Typen des protrahierten Schocks verursachen.

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Radioprotective Effectiveness of Some Serotonin-Like Compounds

Recently it was shown[1] that some sulphur analogues of 5-hydroxytryptamine (serotonin) gave good protection in supralethally irradiated mice. The radioprotective effectiveness of 5-mercaptotryptamine, as well as of so-called 'sulphur analogue of serotonin' [SAS, i.e. 3-(β-aminoethyl)-5-hydroxy-benzo(b)thiophene][2], was close to that of serotonin. Furthermore, 5-methoxytryptamine was found to be also a potent radioprotector in mice[3,4], rats[5] and, to a certain degree, in Rhesus monkies[6]. Therefore it seemed worthwhile to examine 2 other