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STUDIES ON LEUKOKININS—III

PHARMACOLOGICAL ACTIVITIES OF LEUKOKININS M AND PMN*

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Abstract—Studies on the pharmacology of two leukokinins, which do not contain bradykinin as part of their molecules, have shown them to be very active as hypotensive agents and as agents which increase vascular permeability. The leukokinins relax the rat duodenum, indicating that this assay can no longer be considered specific for bradykinin. The importance of these agents in inflammation and other pathological processes is discussed.

LEUKOKININS are kinins produced by white cell enzymes acting on plasma substrates at acidic pH.[1] Isolation and amino acid analysis of two leukokinins, leukokinin-PMN (PMN-kinin) and leukokinin-M whose formation is catalyzed by enzymes present in polymorphonuclear leukocytes and macrophages, have shown that these polypeptides have a composition of 21–25 amino acids and do not contain bradykinin.[1] Nevertheless, leukokinin-PMN has been shown to be active in causing hypotensive responses, contraction of the rat uterus and guinea pig ileum, and relaxation of the rat duodenum.[2] The following is a more detailed report on the pharmacology of the leukokinins PMN and M.

MATERIALS AND METHODS

Assays

Blood pressure assays on rabbits. These assays were carried out using albino rabbits weighing 2·5–3·5 kg which were anesthetized by injection of 30 mg/kg of sodium pentobarbital into the marginal ear vein. After insertion of a tracheal cannula, arterial blood pressure was recorded at the carotid artery by means of a Statham pressure transducer and a Grass model 7 polygraph. Injection of the sample was made into the jugular or femoral vein. Starting mean blood pressure was at 85–100 mm Hg.

Blood pressure assay on the rat. Rats weighing 250 g which were anesthetized with dial-urethane, 0·13 ml/kg were used. Blood pressure was recorded at the carotid

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artery. Injections of agents were made in saline and injected into the jugular vein. Starting mean blood pressure was at 120–130 mm Hg.

Isolated smooth muscle preparations. The guinea pig ileum, rat uterus and rat duodenum were suspended in a 5- or 10-ml vol. tissue bath (Metro Scientific, New York] and bathed in Tyrode's (guinea pig ileum) or de Jalon's solution (rat uterus, rat duodenum] containing 1 μg/ml of atropine sulfate and 1 μg/ml of diphenhydramine. The bathing solution was continuously bubbled with 95% oxygen and 5% carbon dioxide. The temperature of the bathing solution for the guinea pig ileum assay was kept at 35°, the uterus at 24° and the duodenum at 30°. At 24°, the rat uterus is quite stable with little or no spontaneous activity. In our hands this was found preferable to using the tissue at higher temperatures.

The rat uterus in estrus was prepared by injecting virgin rats weighing 200–250 g with 150 μg diethyestilbesterol 18 hr before sacrifice.

Each muscle was placed under 1 g of tension until a stable baseline was obtained. The muscle was readjusted to this tension during the assay of each agonist. Increase or decrease in tension was recorded by a Grass model 7 polygraph using a Grass force-displacement transducer with no spring load.

Bradykinin (Cyclo Chemicals, Calif.) was used as the standard agonist in these assays. Standard bradykinin solutions were made up in 0·001 M *p*-toluenesulfonic acid. Under these conditions bradykinin concentrations of 1 and 2 μg/ml could be maintained for a working day.

Increase in vascular permeability. This was measured by the methods used by Miles and Miles.[3] The abdomens of rabbits (2·5–3·0 kg) and guinea pigs (400–500 g) were shaved with electric clippers and depilated with a commercial depilatory (Nair) 24 hr prior to testing. The Nair was removed by warm water. Thirty min before testing, the rabbits received diphenhydramine (20 mg/kg) or tripelennamine (2 mg/kg) and a 0·5% solution of Evan's blue (1·2 ml/kg) intravenously in the marginal ear vein. When guinea pigs were used, they were given Evan's blue by cardiac puncture 1 hr before testing. The peptides to be tested were dissolved in saline and 0·1 ml was injected intradermally. The diameter and intensity of the resulting lesion (direct observation) was used to estimate the magnitude of the increase in a permeability response as compared to standards of bradykinin.

Pain measurement in dogs. The "dolorimeter" of Van Arman *et al.*,[4] which measures the restraint upon a leg which has been injected with a pain-producing agent, was used to measure pain in dogs. We are indebted to Dr. C. G. Van Arman of the Merck Institute for the use of his laboratory in these measurements.

Leukokinin-PMN and leukokinin-M were obtained by procedures described in the preceding paper.[1]

RESULTS

The molar amounts of the leukokinins as compared to bradykinin necessary to produce equivalent pharmacological actions in a variety of systems are given in Table 1.

Blood pressure. One of the striking properties of the leukokinins is their relatively high potency as a hypotensive agent in the rabbit and the guinea pig. Leukokinin-PMN is especially potent in this regard.

TABLE 1. POTENCY OF LEUKOKININS AS COMPARED TO BRADYKININ*

Assay	Dose of bradykinin to give response (nmoles)	Dose of PMN-kinin to give response (nmoles)	Dose of M kinin to give response (nmoles)
Blood pressure (rabbit)†	1·66 (1·6 μg)	0·48 (1·2 μg)	1·65 (4·7 μg)
Blood pressure (rat)	2·50	0·24	
Uterus (rat)‡	0·008	0·01	0·03
Duodenum (rat)‡	0·016	0·05	0·41
Ileum (guinea pig)‡	0·018	0·24	0·41
Permeability			
(rabbit)	0·41	0·31	0·10
(guinea pig)	0·41	0·31	
Pain (dogs)§	0·82	5·8	

* The doses listed are the average values obtained from three to six separate experiments.
† That amount of agonist which caused a drop of 50 mm Hg.
‡ That amount of agonist which caused a change in tension of 1g in a 5-ml bath.
§ That amount of agonist which caused a change in gait as discussed in Materials and Methods.

Isolated tissues. While the leukokinins and bradykinin are equipotent on the rat uterus, the leukokinins in general are extremely weak as agonists of the guinea pig ileum contraction. The leukokinins differ considerably in their ability to relax the rat duodenum, with leukokinin-PMN being quite potent and leukokinin-M being relatively weak.

Increase in vascular permeability. Kinin formation by white cell enzymes would have important consequences in terms of changes in vascular permeability. Studies on the ability of leukokinin-PMN to increase vascular permeability as measured by the "blueing" response in the rabbit and the guinea pig demonstrated that PMN-kinin is equipotent with bradykinin as far as its ability to cause an increase in vascular permeability. Thus it must be considered a potent agent in this regard. Leukokinin-M is even more potent than leukokinin-PMN, as seen in Table 1.

Pain-producing potency. "Pain" production by PMN-kinin was measured by the method of Van Arman *et al.*,[4] in which a three-legged gait occurs after injection of a pain-producing agent into the synovial space of a knee joint of dogs. In this test, bradykinin in the order of 0·1–1·0 μg causes a response. PMN-kinin, while weaker than bradykinin, still produced a pain response in these animals.

DISCUSSION

Leukokinins have been shown to be potent agents in causing increased vascular permeability and hypotensive changes in blood pressure. They also have some pain-producing potency. Such agents produced by white cell enzymes and tissue enzymes have to be considered as possibly mediating some or many of the responses seen in injury, inflammation, anaphylaxis and shock. Several lines of evidence can be cited to support this.

Steele and Wilhelm,[5] in their studies on the inflammatory reaction, concluded that mediators liberated from polymorphonuclear leukocytes would account for the changes in vascular permeability produced in the inflammatory response seen in their

investigations. Van Arman *et al.*[6] and Vinegar *et al.*[7] are in agreement that white cells are necessary for the occurrence of increased vascular permeability in the model inflammatory systems they employ. Berry *et al.*[8] have pointed out that the acidic conditions which occur in hemorrhagic shock would lend themselves very well to kinin formation by proteases acting at acid pH, a condition now found to be well suited to leukokinin formation. Sensitized lymphocytes releasing such enzymes in the presence of antigen might explain some of the pathology seen in the inflammatory response of anaphylaxis (see Discussion in ref. 1).

Like other mediators such as bradykinin and some prostaglandins, the leukokinins are readily destroyed in the lung[9], thus limiting their systematic activity. Nevertheless, their formation and actions in chronic areas of inflammation where blood supply is poor could be quite long-acting and significant.

One of the major problems in elucidating the pharmacological properties of the leukokinins was to be certain that small amounts of bradykinin-containing peptides were not complexed with them. Several lines of evidence[1] argued strongly against this possibility: the amino acid analyses of both leukokinin-M and leukokinin-PMN showed insufficient phenylalanine for the presence of bradykinin; end-group analysis indicated that the peptides subjected to amino acid analysis were single entities; sequential degradation from the amino-terminal position indicated only by one amino acid was found at each cycle up to seven cycles, indicating purity of the peptides tested; indices of discrimination were dissimilar to those of bradykinin and its analogues.

The finding that leukokinins cause relaxation of the rat duodenum, an assay system long thought to be bradykinin specific, indicates that this system cannot solely be used to identify bradykinin.

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