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PLASMA KALLIKREIN-GENERATING ACTIVITY EVOKED BY RAT PERITONEAL-FLUID MAST CELLS FOLLOWING TREATMENT WITH EPINEPHRINE, 8-BROMO-CYCLIC 3′,5′-GUANOSINE MONOPHOSPHATE OR COMPOUND 48/80

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Abstract—Acting in a dose-dependent fashion, *l*-epinephrine caused rat peritoneal-fluid cells to rapidly deplete rat plasma kininogen *in vitro*; 8-bromo-cyclic 3′,5′-guanosine monophosphate (8-Br-cGMP) behaved similarly; N^6-2′-*O*-dibutyril-cyclic 3′,5′-adenosine monophosphate (di Bu-cAMP) inhibited this effect of epinephrine or 8-Br-cGMP. After fractionation of peritoneal-fluid cells by differential centrifugation, this kininogen-depleting activity was observed only in mast cells; eosinophils, lymphocytes, and monocytes were inactive. Epinephrine-treated mast cells were able to hydrolyze the trypsin substrates N-*p*-toluenesulfonyl-arginine-methyl-ester (TAME) or *N*-benzoyl-arginine-ethyl-ester and to generate the capacity to hydrolyze these substrates in rat plasma; because this activity accompanied kininogen depletion, it was attributed to plasma kininogenase (plasma kallikrein). Diisopropyl-fluorophosphate (DFP) inhibited the mast cell esterase activity toward TAME but did not prevent activated cells from depleting plasma kininogen. Thus, mast cell-bound argininase ester esterase may not have been necessary for the activation of plasma kininogenase. Mast cell heparin, exposed following epinephrine or 8-Br-cGMP treatment, may have been the activator of plasma kallikrein. Unlike DFP, Trasylol [polyvalent bovine proteinase inhibitor (BPTI)] inhibited both mast cell esterase and kininogen-depleting activity. This inhibitor may have acted on mast cells both as a heparin antagonist and as a non-specific esterase inhibitor. Compound 48/80, at concentrations causing 40 per cent release of mast cell histamine, failed to cause mast cells to exhibit the ability to activate plasma kallikrein. At high concentrations it activated the kininogen depleting action of mast cells, but to a lesser degree than did epinephrine or 8-Br-cGMP. These compounds did not release histamine; it may be concluded, therefore, that the ability to activate plasma kininogenase was present in non-histamine releasing mast cells.

Earlier reports from this laboratory [1] have shown that epinephrine treatment leads to partial kininogen depletion and to kinin formation in rat blood *in vivo* and *in vitro*. A pronounced although transitory, enhancement of hydrolysis of substituted arginine esters was also observed in the plasma of epinphrine-treated rats [2]. Catecholamine activation of the kallikrein–kinin system may occur in patients with pheochromacytoma, in whom bursts of abnormally high adrenomedullary discharges are accompanied by increased urinary excretion of kinin-forming enzyme [3]. Epinephrine-induced flushes in carcinoid-tumor-bearing patients believed to be due to kinin, are accompanied by increased circulating levels of arginine ester hydrolase activity, possibly kallikrein [4]. Kinin release is thought to occur in experimental traumatic shock [5], a condition which may involve adrenomedullary discharge, as well as in acute pulmonary edema evoked by epinephrine in the rat [6].

In the earlier report [1], it was shown that plasma kininogen activity could be decreased by epinephrine in rat whole blood, but not in rat cell-free plasma. Although the cellular elements that were required for this epinephrine effect were not fully classified, it was shown that they had to be neutrophils, basophils, or both [7]. Basophils exist in low numbers in rat blood [8]. The probability that they were mediators of the observed changes received indirect support from results showing that, following epinephrine, rat peritoneal-fluid cell suspensions, which do not contain neutrophils acquired the ability to cause kininogen depletion and kinin production upon addition to rat plasma [9].

The first objective of the present work was to investigate which of the cells present in rat peritoneal fluid suspensions exhibit plasma kallikrein activating action following epinephrine treatment.

In a more recent report it has been shown that, following activation by epinephrine, isolated rat peritoneal-fluid mast cells exhibit hydrolytic activity toward substituted amino acid esters that is inhibited by DFP* [10]. In the present work, the participation

* Abbreviations: DFP, diisopropyl-fluorophosphate; BAEE, *N*-benzoyle-arginine-ethyl-ester; TAME, *N*-*p*-toluenesulfonyl-arginine-methyl-ester; BSA, bovine serum albumin; di-Bu-cAMP, N^6-2′-*O*-dibutyril-cyclic 3′,5′-adenosine monophosphate; 8-Br-cGMP, 8-bromo-cyclic 3′,5′-guanosine monophosphate; TCA, trichloroacetic acid and BPTI, polyvalent bovine proteinase inhibitor (Trasylol).

of this hydrolytic activity in plasma kallikrein activation by epinephrine-treated peritoneal-fluid cells was estimated by comparing such mast cell activity toward plasma prior to, and following, DFP treatment. The non-specific bovine proteinase polyvalent inhibitor (BPTI) Trasylol [11] had been shown to block epinephrine-induced kininogen depletion in rat blood [7]. To better understand the action of this important inhibitor of kinin formation, its effects on the activation of peritoneal-fluid mast cells by epinephrine and on plasma kallikrein activity have been examined separately.

cAMP is the generally acknowledged second messenger of epinephrine effects on cells or cellular systems [12], but it has been postulated that cGMP also may mediate some cellular actions of the catecholamine [13]. In a previous report [14], we showed that kininogen depletion evoked by epinephrine in rat blood is prevented by the lipid-soluble, phosphodiesterase-resistant analogue of cAMP, diBu-cAMP. 8-Br-cGMP was not only only devoid of such action but, on the contrary, slightly potentiated the effect of epinephrine and reversed the inhibition by diBu-cAMP. A similar reciprocal relationship between these nucleotide derivatives has been shown regarding the activation of mast cell hydrolases [10]. In view of these observations, the effects of diBu-cAMP and 8-Br-cGMP were also examined in connection with epinephrine-induced activation of plasma kallikrein by rat peritoneal-fluid cells.

It had been shown that concentrations of compound 48/80 sufficient to release 40 per cent of mast cell histamine failed to generate in mixed rat peritoneal-fluid cells the ability to activate plasma kallikein. Yet, partly histamine-depleted cells could still be normally activated by subsequent epinephrine treatment [7]. This dichotomy between histamine releasing, and plasma kallikrein activating, ability of rat peritoneal-fluid cells has been further investigated.

MATERIALS AND METHODS

Wistar strain (180–250 g) male rats were used. Plasma from Nembutal (30 mg/kg) anesthetized animals was separated by centrifugation (20 min) of blood drawn by cardiac puncture in the presence of 0.2% sodium oxalate with the avoidance of contact with glass surfaces. Peritoneal-fluid cells were collected by centrifugation for 5 min at 900 *g* of 10 ml washings of the peritoneal cavity of the animal with Krebs–Ringer phosphate buffer, pH 7.3. Mast cells were separated by density fractionation using 38% BSA [15]. After activation by epinephrine or other compounds, aliquots of cell suspensions were incubated with plasma at 37° under air in a Dubnoff metabolic incubator at a shaking cycle of 125/min. Unless otherwise stated, details of activation and incubation conditions were as described in Fig. 1. It is worth noting that no release of histamine occurred following incubation of peritoneal-fluid cells with 2:3 diluted rat plasma, i.e. the condition employed in the present study. Kininogen remaining in plasma after incubation with control or activated cells was estimated by bioassay of kinin released from it by trypsin after acid plus heat denaturation [9]; a bradykinin triacetate standard and the atropinized, antihistamine-treated guinea pig ileum were employed. Controls in which plasma was incubated in buffer were considered to contain 100 per cent kininogen. Esterolytic activity was determined by allowing 300 μl of activated or control cell suspensions in buffer, or 300 μl of such cell suspensions in plasma, to act on 900 μl of a 10 mM solution of ester substrate in 0.15 M Tris buffer, pH 7.8, for 30 min at 37°. Reactions were terminated by adding 10% TCA to a final concentration of 4%. Non-hydrolyzed ester was determined using the colorimetric procedure recommended by Brown [16]. Values of hydrolysis observed following incubation of plasma and substrate in buffer were substracted from corresponding experimental values. Controls in which

Table 1. Mast cell origin of plasma kallikrein generating activity evoked by epinephrine or compound 48/80 in rat peritoneal fluid cells*

Source	Cells in incubates Content per ml × 10^{-3} Mast cells	Non-mast cells	Activity Treatment	Kininogen (μg/ml plasma)	% Lost
Non-fractionated peritoneal fluid	294 ± 35	4587 ± 230	Buffer	2.00 ± 0.00	0
			Epinephrine	1.00 ± 0.08†	50
			48/80	1.64 ± 0.06†	18
Albumin layer	317 ± 35	94 ± 13	Buffer	1.82 ± 0.11	9
			Epinephrine	0.84 ± 0.15†	62
			48/80	1.48 ± 0.16†	26
Interfacial layer	6 ± 6	4538 ± 230	Buffer	2.00 ± 0.00	0
			Epinephrine	2.00 ± 0.00	0
			48/80	2.00 ± 0.00	0

* After fractionation [10], cells were incubated for 5 min with epinephrine (2.7×10^{-6} M) or compound 48/80 (5.0 μg/ml); 0.1 ml of each incubate was added to 0.2 ml of rat plasma and reincubated for 5 min at 37°. Cell concentration shown refer to those present in these mixtures. Results refer to averages of four experiments.

† Statistically significant effect ($P < 0.05$) of epinephrine or compound 48/80, compared to control. Comparisons were made by Student's *t*-test on paired samples.

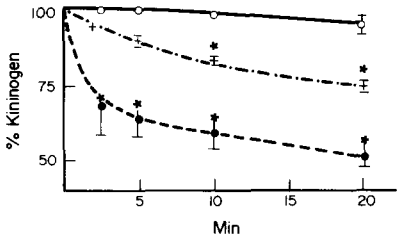

Fig. 1. Time course of appearance of plasma kininogen depleting activity in rat peritoneal-fluid cells incubated in 2.7×10^{-6} M epinephrine (●), 5.0 μg/ml of compound 48/80 (+), or buffer (○). After activation, 100 μl of this medium, containing an average of 10^6 cells, 3–6% of which were mast cells, was incubated with 200 μl of plasma for 5 min at 37°. Results are averages of four experiments. Vertical bars indicate respective standard errors of the mean. An asterisk (*) indicates a statistically significant ($P < 0.05$) effect of epinephrine or compound 48/80.

substrate alone was incubated in buffer were considered to contain starting amounts of substrate.

Histamine release was estimated by bioassay on the guinea pig ileum [17]. Cells, stained with Giemsa or toluidine blue, were counted in the Neubauer chamber.

Drugs. *l*-Epinephrine, compound 48/80, diBu-cAMP, 8-Br-cGMP BAEE, TAME, BSA (fraction V), trypsin (Type III), and bradykinin triacetate were obtained from the Sigma Chemical Co., St. Louis, MO. DFP was obtained from the Aldrich Chemical Co., Milwaukee, WI, through the kindness of Dr. M. E. Webster. Stock solutions of epinephrine (1 mg/ml) were prepared by dissolving the amine in 1 M HCl and adding distilled water to a final concentration of 1 mg catecholamine/ml (pH 3–4).

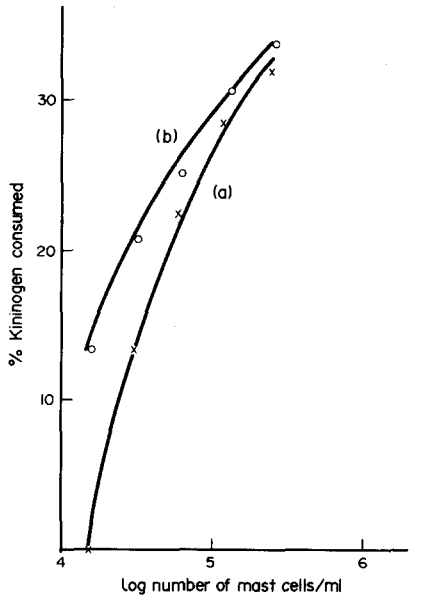

Fig. 2. Kininogen depletion caused by various concentrations of rat peritoneal-fluid cells activated for 5 min in 2.7×10^{-6} M epinephrine and incubated for 5 min (line a), and 10 or 20 min (line b), with rat plasma. Each point refers to averages obtained from two experiments; identical results were obtained after 10-or 20-min incubation.

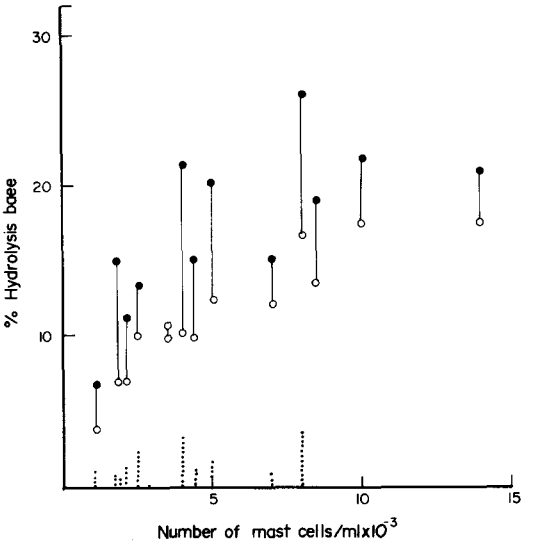

Fig. 3. Relationship between mast cell concentration and esterolysis of BAEE in epinephrine activated rat peritoneal-fluid cell suspensions incubated in buffer (○) or in plasma (●). Full lines represent increases of esterolytic activity caused by the use of plasma as the incubation medium. Dotted lines represent increases of esterolytic activity observed in the presence of plasma in controls containing cells not exposed to epinephrine. Vertical lines join results obtained with cells from the same rat.

Appropriate dilutions in saline, pH 5–6, were prepared just prior to use. DFP was dissolved in methanol and subsequently diluted to the desired concentration with buffer. Trasylol (polyvalent antiprotease from bovine tissue) was obtained from Bayer A. G., Leverkusen, GFR.

RESULTS

Figure 1 illustrates the time course of activation by epinephrine or compound 48/80 of the ability of rat peritoneal-fluid cells to deplete plasma kininogen. Control cells, incubated for up to 20 min in buffer, caused negligible changes. Cells pre-incubated for 2.5 min in 2.7×10^{-6} M epinephrine evoked, following addition to plasma, conspicuous loss of kininogen; longer incubation did little to increase the

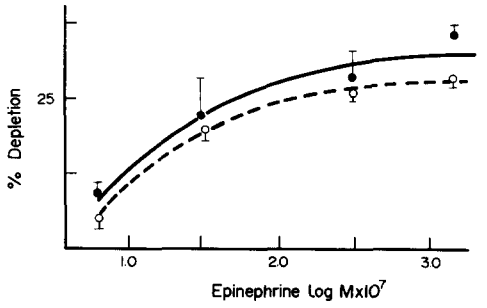

Fig. 4. Depletion of plasma kininogen (●——●) and of BAEE (○ - - - ○) caused by rat peritoneal-fluid mast cells exposed to various concentrations of epinephrine. Results are averages of four experiments; vertical bars indicate the respective standard errors of the mean.

Table 2. Effects of DFP on TAME esterase and plasma kallikrein activities evoked by epinephrine in peritoneal-fluid cells of the rat*

Incubation	Activity (% depleted) TAME	Kininogen
Buffer	2.6 ± 1.0	3.0 ± 1.9
Epinephrine (2.7×10^{-6} M)	20.2 ± 3.4†	42.0 ± 4.7†
DFP (5×10^{-3} M) + epinephrine	4.9 ± 1.4‡	35.8 ± 6.4†

* Peritoneal-fluid cells, suspended in Krebs–Ringer phosphate buffer, were incubated for 15 min at 37°. After triple centrifugation and washing, they were assayed for activity toward TAME in buffer and toward kininogen, in plasma. Results are averages of six experiments.

† Statistically significant ($P < 0.001$) effect of epinephrine compared to control.

‡ Statistically significant ($P < 0.01$) effect of DFP compared to effect of epinephrine alone.

activity. Pretreatment of cells with compound 48/80 led to smaller activation. Previous observations [1] had shown that neither epinephrine nor 48/80 affected kininogen in plasma in the absence of cells. To determine the origin of the activity exhibited by the peritoneal-fluid cells, they were separated into a mast cell-enriched and a mast cell-deprived fraction by density barrier centrifugation, using bovine serum albumin. Table 1 shows that mast cells, collecting in the albumin layer after centrifugation, were capable of causing kininogen loss following activation by epinephrine or compound 48/80. Other cells retained on top of the albumin layer, were unresponsive to activation by epinephrine or 48/80, though it has been shown [10] that they were quantitatively recovered after fractionation. Kininogen depletion was a rapid process and was dependent on the number of mast cells present. Figure 2 shows that when 10^5 cells, activated by 5 min contact with epinephrine, were incubated in 1 ml of rat plasma, loss of kininogen was essentially the same after 5, 10 or 20 min. On the other hand, when a 10-fold lower number of mast cells (10^4/ml) was added to plasma, kininogen loss required more than 5 min to become detectable.

Peritoneal-fluid cells exposed to epinephrine are able to hydrolyze substituted arginine esters such as BAEE or TAME [10]. When these cells were incubated with rat plasma, additional esterolytic activity was generated. This is illustrated in Fig. 3, where hydrolysis of BAEE is plotted as a function of the concentration of mast cells in the incubates. It can be seen that activity was invariably increased by the presence of plasma. When the differences between corresponding experimental points, i.e. hydrolysis in the presence, minus hydrolysis in the absence, of plasma were averaged, it became apparent that the latter had caused the hydrolysis of 5.75 ± 0.87 per cent substrate, corresponding to a loss of 15.8 ± 2.8 μmoles BAEE/ml plasma during the 30-min incubation period; this effect was statistically significant at ($P < 0.001$). Cells not exposed to epinephrine also induced slight esterolytic activity in plasma, statistically significant at $P < 0.02$. Confirming previous results [10], activity in control cells incubated with BAEE in the absence of plasma was low or absent.

Variation of the concentration of epinephrine in the peritoneal cell suspensions led to parallel changes in the esterolysis of BAEE and in the depletion of plasma kininogen respectively (Fig. 4). In spite of this, further experiments suggested that the relationship between these activities was coincidental rather than causal. As shown previously [10], the arginine ester hydrolyzing activity that is developed in mast cells by epinephrine is inhibited by DFP: Table 2 shows that, in spite of this, DFP did not block the activity of the cells toward plasma kininogen. Thus it appears that the esterolytic activity of mast cells that was generated by epinephrine was

Table 3. Sensitivity to BPTI (Trasylol) of TAME esterase and of plasma kallikrein generating activity evoked by epinephrine in peritoneal fluid cells of the rat*

Incubation	Activity (% depleted) TAME	Kininogen
Epinephrine (2.7×10^{-6} M)	20.6 ± 1.0 (4)	27.2 ± 3.2 (6)
BPTI (10 KIU/ml)† + epinephrine	3.8 ± 0.8‡(4)	0.8 ± 0.8‡(6)

* Peritoneal-fluid cells, suspended in Krebs–Ringer phosphate buffer, were incubated for 5 min at 37°. After a single washing and centrifugation, the effects of the cells on esterolysis of TAME in buffer and on depletion of kininogen in plasma were assayed. Figures in parentheses refer to the numbers of experiments.

† Kallikrein inhibiting units [11].

‡ Statistically significant ($P < 0.001$) effect of BPTI compared to controls incubated in the presence of epinephrine.

Table 4. Effect of BPTI (Trasylol) on kininogen depletion in rat plasma evoked by epinephrine activated peritoneal mast cells*

Controls	32.0 ± 3.0
BPTI (10 KIU/ml)	19.1 ± 4.5

* Peritoneal-fluid mast cells were incubated for 5 min at 370° with 2.7×10^{-6} M epinephrine and then were added to rat plasma in the presence or absence of BPTI and incubated for another 2 min at 37°. Cells not activated by epinephrine (not shown) did not alter kininogen levels in plasma regardless of the presence of Trasylol. Results are averages of seven experiments ± S.E.M. The $P < 0.005$ that the difference between the two values would occur by chance (Student's *t*-test).

not required for the activation of the plasma kininogen depleting system. The action of DFP contrasts with that of the bovine polyvalent antiprotease (BPTI), Trasylol. As indicated in Table 3, this substance was able to inhibit both mast cell arginine-ester hydrolase and kininogen-depleting activity. Trasylol appears to act predominantly on cells; Table 4 shows that, when mast cells that had been pre-activated by epinephrine were added to Trasylol-containing rat plasma, their effect on kininogen was decreased only partially.

It had been shown [10] that arginine-ester esterase activity could be generated in the presence of mast cells by the putative mediator of certain effects of epinephrine, cGMP, used in the form of its lipid-soluble derivative, 8-Br-cGMP. Table 5 shows that this compound was an effective activator of the kininogen-depleting ability of rat peritoneal-fluid mast cells. In contrast, the lipoidic cAMP substitute dibutyryl-cAMP was not only incapable of activating peritoneal mast cells but actually inhibited their activation by either 8-Br-cGMP or epinephrine. Such inhibition had been observed previously in relation to the BAEE- and BTEE-hydrolyzing activities of rat mast cells generated by either 8-Br-cGMP or epinephrine [10]; it suggests a common origin for the processes by which the esterolytic and kininogen-depleting properties of mast cells are unfolded.

Table 5. Effects of 8-Br-cGMP, epinephrine, and diBu-cAMP on generation of plasma kininogen-depleting activity in rat peritoneal-fluid cells*

Incubation	Kininogen depletion (%)
Buffer	0.0
8-Br-cGMP (10^{-4} M)	45.0 ± 5.0
Epinephrine (2.7×10^{-6} M)	50.0 ± 5.8
DiBu-cAMP (10^{-4} M)	0.0
8-Br-cGMP + diBu-cAMP	21.3 ± 6.3†
Epinephrine + diBu-cAMP	3.0 ± 1.0†

* Peritoneal-fluid cells, suspended in Krebs–Ringer phosphate buffer, were incubated for 5 min at 37°; after centrifugation and one washing, they were assayed for activity toward kininogen in plasma. Results refer to averages of three experiments.

† Statistically significant ($P < 0.05$) inhibitory effect of diBu-cAMP compared to samples incubated, respectively, in the presence of 8-Br-cGMP or of epinephrine alone.

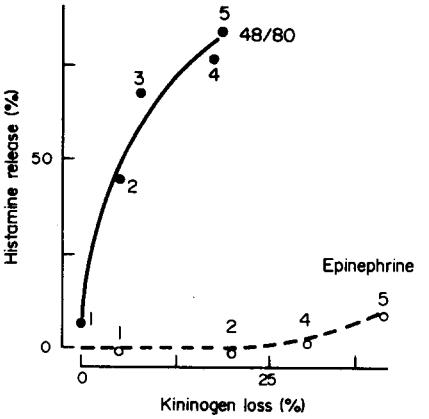

Fig. 5. Histamine release and plasma kininogen depletion evoked by rat peritoneal fluid cells following a 10-min exposure to various concentrations of epinephrine (○ - - - ○) or compound 48/80 (●——●); the latter are expressed in terms of the *p*-methoxy phenethyl methylamine monomer content of 48/80. Concentrations 1:0.54 × 10^{-6}M; 2:2.7 × 10^{-6}M; 3:5.4 × 10^{-6}M; 4:27 × 10^{-6}M and 5:135 × 10^{-6}M. Results are averages of four experiments. Controls incubated in the absence of activators did not exhibit measurable histamine release or kininogen depletion.

The capacity to release histamine and the capacity to respond to epinephrine are not necessarily concomitant events in rat mast cells. This is illustrated in Fig. 5, which shows that, following activation by epinephrine, peritoneal-fluid cells were able to cause an appreciable decrease in plasma kininogen without undergoing loss of histamine. Compound 48/80-treated cells, on the contrary, released appreciable amounts of histamine without causing kininogen-depletion; both events did, however, appear to occur simultaneously at higher concentrations of compound 48/80.

DISCUSSION

The present report confirms preliminary evidence suggesting that plasma kininogen-depleting and plasma kinin generating activities arising after epinephrine treatment of rat peritoneal cavity cells *in vitro* originate from mast cells [9]. Other loose peritoneal cells—viz lymphocytes, eosinophils and monocytes—were not necessary for this activity. The kininogen-depleting activity was maximal within 5 min after exposure of cells to epinephrine; its generation was slower after exposure to compound 48/80. The rapid response of mast cells contrasts with the slow development of another reported form of cell linked kinin-generating activity, namely, that emanating from acid-treated PMN leukocytes or lymphocytes, which is complete after 12–15 hr [18]. Rapid, spontaneous generation of kininogen-depleting activity has, however, been reported to occur following incubation of human plasma with human granulocytes [19]. The relationship between this event and the activation of the kininogenase-gen-

erating activity of mast cells by epinephrine remains to be established; it may be meaningful that both responses have a rapid onset, often an important requirement of naturally occurring regulatory processes. Although the reversibility of mast cell activation by epinephrine has not been studied, it has been noted that accompanying changes of cell size and granular density were comparatively short lived and could be repeated by renewed treatment with the catecholamine [17]. Mast cell response to epinephrine was shown to be influenced by active analogues of cGMP and cAMP; their actions appeared to be antagonistic and may support the Ying–Yang, dualistic hypothesis of cyclic nucleotide control of cell functions [20].

The incubation of epinephrine-activated mast cells, which possess BAEE-esterase activity, with rat plasma led to enhanced BAEE-esterase activity. The activity in plasma could be responsible for the kininogen depletion and could represent plasma kallikrein. In epinephrine-activated mast cells, both the generation of cell-bound BAEE-esterase [10] and of plasma kininogen-depleting action (this report) have similar characteristics of speed, dependence on catecholamine concentration, sensitivity to cyclic nucleotides, and lack of correlation with histamine release. This similarity was expected to extend to blockade by DFP and to implicate mast cell serine esterases in plasma kininogen depletion. It was surprising to find this not to be so—DFP-treated cells showing practically undiminished kininogen-depleting capacity in spite of extensive loss of direct activity toward TAME. Thus, cell-bound arginine ester hydrolase activity may not be required for the effect of epinephrine-activated mast cells on plasma kininogen. While it is, of course, not impossible that DFP-resistant esterases of the mast cell are involved, the following alternative hypothesis is presented to explain these findings. Rat mast cells contain the negatively charged macromolecular derivative of the sulfated mucopolysaccharide, heparin [21]. The ability of sulfated macromolecules to activate plasma kininogenase via the Hageman factor-dependent pathway is well known [22–25]. It is improbable that DFP interacts directly with heparin; mast cells, however, may employ this mucopolysaccharide to bind, and subsequently to activate, plasma kininogenase. In our experiments (Table 2), washing of epinephrine activated cells did not eliminate the kininogenase activating effect. It is possible, therefore, that cell-bound heparin, exposed to the external medium following epinephrine activation becomes a nucleus of interaction for Hageman factor, pre-kallikrein, and kininogen contained in the plasma reaching the cell. Activated mast cells would then be acting somewhat like negatively charged surfaces of the kaolin type, which promote kinin production by contact activation. It is interesting to note in this connection that kininogen, a protein having a high isoelectric point, is bound by kaolin [26]. Studies attempting to demonstrate similar binding by epinephrine activated mast cells are in progress at present in this laboratory. Others have postulated that kininogen depletion in human plasma with granulocytes occurs via activation of Hageman factor [27, 28]; it would be remarkable if heparin-like acid mucopolysaccharide constituents of basophilic granulocytes [29–32] were found to be responsible for kininogenase activating properties, not only of sessile mast cells, but also of circulating basophils.

In contrast to DFP, the bovine tissue polyvalent antiprotease (BPTI, Trasylol) was able to block both the BAEE-esterolylic and kininogen-depleting effects of epinephrine activated mast cells. Besides reacting with many proteases [11], Trasylol binds strongly to heparin *in vitro* [33] and to the mast cell granule, which is a heparin storage site, *in vivo* [34]. Such dual reactivity may explain why, in contrast to DFP, Trasylol blocked not only the mast cell tryptic esterase but also their putatively heparin mediated kininogen-depleting activity. Natural localization of Trasylol in mast cells has been demonstrated [35]; perhaps dissociation of heparin–Trasylol complexes [33] is involved in the mechanism by which mast cells acquire the kininogenase activating property. Trasylol was only partially effective in inhibiting kininogen-depleting activity evoked by normally activated mast cells in rat plasma. This result contrasts with the marked blockade observed when this inhibitor was present during the cell activation phase and may reflect the low sensitivity to Trasylol of rat plasma kallikrein compared with that of other species, including man [11]. This had been noted previously in studies showing that, in contrast to the kininogen effect evoked by epinephrine in rat blood, cellulose sulfate activation of rat plasma kallikrein, a process not requiring cells, was insensitive to Trasylol [7].

Although less effective than epinephrine, compound 48/80 activated esterolytic and kininogen-depleting activities of mast cells. Treatment of rats with compound 48/80 leads to histamine release and loss of plasma kininogen [36]. The present study indicates that these phenomena may indeed occur concomitantly at sufficiently high concentrations of compound 48/80. Nevertheless, they do not appear to be associated: neither epinephrine nor 8-Br-cGMP released histamine from mast cells when causing activation of kininogen-depleting action. At low concentrations, compound 48/80 released almost half of mast cell histamine without inducing kininogen-depleting activity. These findings recall reports showing that, in immunologically challenged human basophils, histamine release and generation of kinin-forming activity, although simultaneous events may represent selective processes originating at different granular structures of the basophil [37, 38]. Epinephrine and 8-Br-cGMP treatments are means of selectively generating kininogen-depleting activity in mast cells. They may be useful experimental models for the study of basophil- or mast cell-dependent exposure of kininogenase (kallikrein) activators in blood or tissues.

The preferential perivascular [39], and subendothelial [40], locations of mast cells support the assumption that influences regulating microvascular flow may emanate from these cells. Catecholamine or cGMP-mediated production of vaso-active kinins in close vicinity to reactive blood vessels may be one means of attaining this objective. Antigen-induced activation of kinin-forming systems by sensitized mast cells, as yet an untapped field, may be another.

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