Chem. Pharm. Bull.
33(2) 642—646 (1985)

Activation of Hyaluronidase by Metallic Salts and Compound 48/80, and Inhibitory Effect of Anti-allergic Agents on Hyaluronidase

HISAO KAKEGAWA, HITOSHI MATSUMOTO and TOSHIO SATOH*

School of Pharmacy, Tokushima University of Arts and Science, Yamashiro-cho, Tokushima 770, Japan

(Received April 18, 1984)

Modulation of hyaluronidase activities by various compounds which are closely related with the degranulation of the mast cells was studied. It was found that compound 48/80, a non-specific histamine releaser from mast cells, activated hyaluronidase strongly. Hyaluronidase activated by $CaCl_2$ or compound 48/80 was inhibited by anti-allergic agents such as disodium cromoglycate (DSCG) or tranilast. On the other hand, tranilast greatly enhanced the activity of the hyaluronidase activated by NaCl. The inhibitory effects of these anti-allergic agents on the activation of the inactive hyaluronidase were shown to be stronger than those on activated hyaluronidase.

Since the hyaluronidase activity was modulated by compounds closely associated with allergic phenomena, it is suggested that the hyaluronidase might be present as one of the target enzymes of the influent calcium ions in the mast cells and might directly control the degranulation.

Keywords——allergy; calcium ion; compound 48/80; hyaluronidase; DSCG; tranilast; anti-allergic agent

Allergy can be divided into various types, I, II and III (immediate-type hypersensitivity) and IV (delayed-type hypersensitivity), according to the initial mechanisms, and the methods used for therapy should be quite different. The type I allergy, which produces such symptoms as asthma, hay fever and rhinitis, is the most common. The pathological mechanism of type I allergy has been explained[1] in terms of the histamine-releasing reaction and the degranulation of mast cells. The degranulation of the mast cells takes place following an immunological stimulus in which the antigen IgE antibody reaction on the membrane of the mast cells predominates and is thought to require the presence of extracellular calcium ions. It is considered that this degranulation is induced by the flow of the calcium ions into the cells through the calcium channels, and this activates certain enzyme reactions. The mechanism of the degranulation triggered by extracellular calcium ions is still far from being known in detaile, and the intracellular series of enzyme reactions has been clarified only to a very limited extent.

Compound 48/80, a polycondensate of *N*-methyl-*p*-methoxyphenethylamine and formaldehyde, is an inflammatory substance which is known to release histamine, inducing an extensive degranulation of the mast cells without mediation by the antigen IgE antibody reaction.

On the other hand, DSCG (disodium cromoglycate)[2] and tranilast (*N*-3′,4′-dimethoxycinnamoyl anthranilic acid),[3] used in practice as anti-allergic agents, are considered to be mast cell stabilizers, the mechanism of their medicinal effects is presumed to involve inhibition of the degranulation of mast cells caused by the antigen IgE antibody reaction. Although the modes of action of compound 48/80, DSCG and tranilast in the mast cells have not been fully explained as yet, it is possible that effects on enzymes may be involved, as well as interaction between the receptors in the mast cells and these drugs.

Some enzymes are known to be present in mast cells, and to be probably associated with the liberation of histamine from the mast cells. Examples of these are phospholipase A_2,[4] chymotrypsin-like enzyme,[5] adenylcyclase[6] and phosphodiesterase.[6] However, little has been reported on the interaction between these known enzymes and such compounds as compound 48/80, DSCG and tranilast.

Since it is known[7] that hyaluronidase, one of the enzymes involved in the inflammatory reaction, usually exists in an inactive form and is activated *in vivo* by metal ions, including calcium ions, and also[8] that the substrate, hyaluronic acid, is present in substantial quantities in mast cells, we considered that hyaluronidase might be a target enzyme of the calcium ions in the degranulation of mast cells, and thus we attempted to investigate how hyaluronidase activity might be modulated by drugs such as compound 48/80, DSCG and tranilast in association with the histamine-liberating reaction.

Results and Discussion

Activation of hyaluronidase by various metal salts was tested in order to compare the activation profile with that of an organic activator, compound 48/80. Figure 1 shows the enzyme activity in terms of the optical density at 585 nm when NaCl, KCl, $CaCl_2$ and $MgCl_2$ were used as activators. These metal salts did cause hyaluronidase activity to appear. Moreover, the activating actions were different between monovalent NaCl and KCl and divalent $CaCl_2$ and $MgCl_2$. With the latter metals, the hyaluronidase-activating ability decreased gradually from a concentration of near 0.02 M, and hyaluronidase was scarcely activated at near 0.16 M, whereas with the former metals the activation was unchanged at 0.16 M. In previous papers,[9a,b] these phenomena could not be observed because 0.15 M NaCl was always used as a stabilizer when the influence of other metallic salts was tested.

The hyaluronidase-activating action of compound 48/80 is shown in Fig. 2; the effect was concentration-dependent even in the absence of metal salts. Moreover, the activating ability of the compound was greater than that of the metal salts. This is a first report that the activity of an enzyme(s) is modulated by compound 48/80.

Inhibition of hyaluronidase by DSCG was tested in the presence of 2.5 mM $CaCl_2$, 0.15 M NaCl and 0.1 mg/ml compound 48/80 as activators of the enzyme. Since there are two types of hyaluronidase, active and inactive, the inhibition of activated hyaluronidase and the inhibition of the activation of inactive hyaluronidase were both studied. Figure 3 shows the

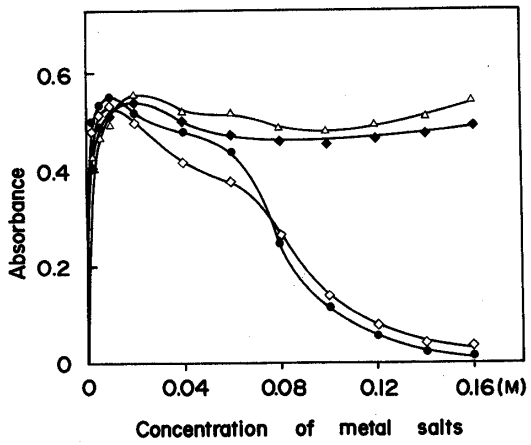

Fig. 1. Activation of Hyaluronidase by Metal Salts

◆—◆, NaCl; △—△, KCl; ●—●, $CaCl_2$; ◇—◇, $MgCl_2$.

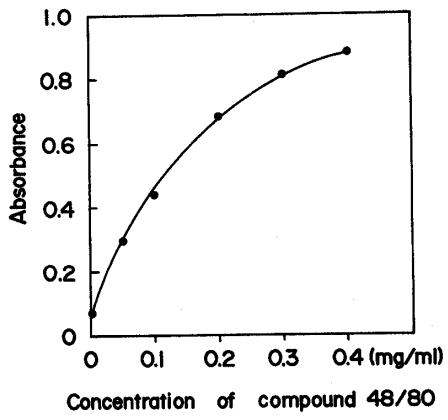

Fig. 2. Activation of Hyaluronidase by Compound 48/80

●—●, compound 48/80.

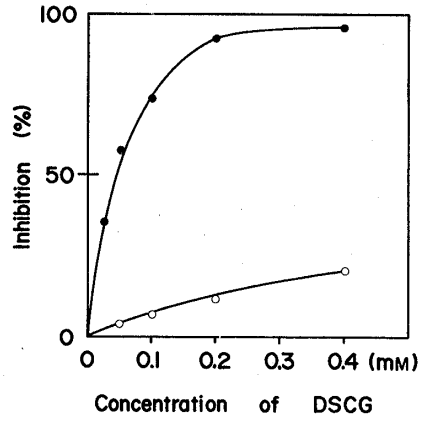

Fig. 3. Inhibition of Hyaluronidase by DSCG

●—●, inhibition of activation of inactive hyaluronidase; ○—○, inhibition of activated hyaluronidase; (activator: 0.15 M NaCl).

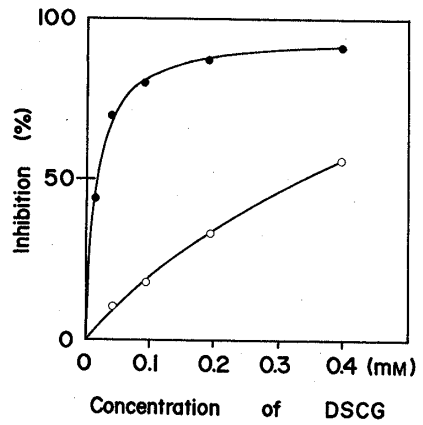

Fig. 4. Inhibition of Hyaluronidase by DSCG

●—●, inhibition of activation of inactive hyaluronidase; ○—○, inhibition of activated hyaluronidase; (activator: 2.5 mM $CaCl_2$).

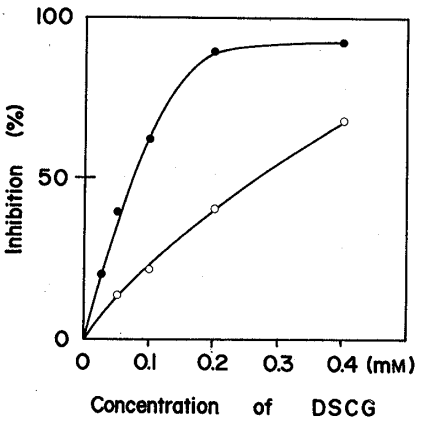

Fig. 5. Inhibition of Hyaluronidase by DSCG

●—●, inhibition of activation of inactive hyaluronidase; ○—○, inhibition of activated hyaluronidase; (activator: 0.1 mg/ml compound 48/80).

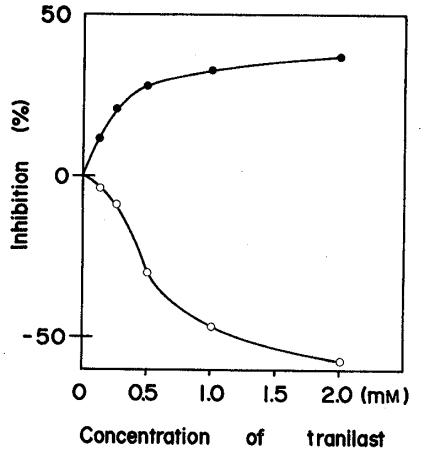

Fig. 6. Inhibition of Hyaluronidase by Tranilast

●—●, inhibition of activation of inactive hyaluronidase; ○—○, inhibition of activated hyaluronidase; (activator: 0.15 M NaCl).

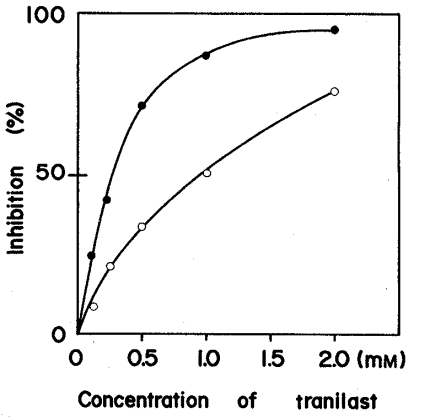

Fig. 7. Inhibition of Hyaluronidase by Tranilast

●—●, inhibition of activation of inactive hyaluronidase; ○—○, inhibition of activated hyaluronidase; (activator: 2.5 mM $CaCl_2$).

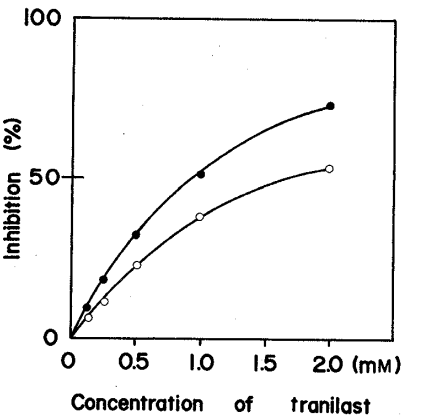

Fig. 8. Inhibition of Hyaluronidase by Tranilast

●—●, inhibition of activation of inactive hyaluronidase; ○—○, inhibition of activated hyaluronidase; (activator: 0.1 mg/ml compound 48/80).

inhibitory effects of DSCG on both active hyaluronidase and the activation stage of inactive hyaluronidase. DSCG showed concentration-dependent inhibitory actions in both cases, but inhibited the activation of the inactive hyaluronidase more strongly. Figures 4 and 5 show the results for DSCG when $CaCl_2$ and compound 48/80 were used as activators. In both cases, DSCG inhibited the activation of inactive hyaluronidase more strongly than the activity of activated hyaluronidase.

Next, the anti-hyaluronidase activity of tranilast was studied. Tranilast is an anti-allergic agent developed in Japan for oral administration and it is considered, like DSCG, to inhibit the degranulation of mast cells due to the antigen-antibody reaction. In this experiment, as with DSCG, the two inhibitory actions were tested. In contrast to the case of DSCG, the inhibitory modes were radically different according to the kind of activating agents. Figure 6 shows the results obtained when NaCl was used as the activating agent. Tranilast inhibited only the activation of inactive hyaluronidase, whereas it enhanced the activity of NaCl-activated hyaluronidase. However, the inhibitory effect on the activation of inactive hyaluronidase was only very weak. Figure 7 shows the hyaluronidase-inhibitory effects of tranilast when $CaCl_2$ was used as the activating agent. In this case, in contrast with the case of NaCl as the activating agent, strong inhibitory action was noted not only on the activated hyaluronidase but also on the activation of inactive hyaluronidase. As with DSCG, tranilast inhibited the activation of inactive hyaluronidase more strongly than the activity of the activated enzyme.

Figure 8 shows the results obtained when compound 48/80 was used as the activating agent. In this case, too, tranilast showed both inhibitory effects, but they were drastically weakened in comparison with the inhibitory action observed when $CaCl_2$ was used as the activating agent. These results show that compound 48/80 and calcium ions, which are closely associated with the degranulation process from mast cells, possess hyaluronidase-activating action. Further, the anti-allergic agents, DSCG and tranilast, which act by inhibiting degranulation of mast cells, were found to inhibit the activity of hyaluronidase activated by compound 48/80 or calcium ions and also to inhibit the activation of inactive hyaluronidase.

So far, most investigators have used 0.15 M NaCl as the activating agent for measurement of hyaluronidase activity and they have only studied the action of hyaluronidase inhibitors with NaCl-activated hyaluronidase. However, the present results show that the selection of activating agents is very important. In particular, we found that the inhibitory effect of anti-allergic agents on $CaCl_2$-activated hyaluronidase was more closely associated with their inhibitory behavior on the degranulation of mast cells induced by antigen–antibody reaction. Therefore, it is preferable to use calcium ions rather than sodium ions as the activating agent when the inhibition of hyaluronidase is to be studied.

At any rate, the above results may suggest that hyaluronidase is present[10] as one of the target enzymes directly controlling the degranulation of mast cells. Therefore, if strongly hyaluronidase-inhibitory substances can be discovered by using calcium ions as the activating agent, they may be expected to have anti-allergic activity and may provide a basis for developing new anti-allergic agents.

Experimental

Assay of Hyaluronidase Activity——Hyaluronidase activity was determined by the Morgan–Elson method[11] as modified by Davidson *et al.*[9a] after incubation of 340 NFunit/ml of hyaluronidase with 0.6 mg/ml hyaluronic acid potassium salt at 37 °C for 40 min in 0.1 M acetate buffer of pH 3.5. Calcium chloride (2.5 mM), sodium chloride (0.15 M) and compound 48/80 (0.1 mg/ml) were used as activators of hyaluronidase.

Inhibition of Activated Hyaluronidase by Anti-allergic Agents——The inhibition of activated hyaluronidase by anti-allergic agents was determined by the above method after incubation at 37 °C for 20 min in 0.1 M acetate buffer of pH 3.5 with the anti-allergic agent in the presence of hyaluronidase which had been activated by preincubation with

the activator at 37 °C for 20 min in the same buffer.

Inhibition of Hyaluronidase Activation by Anti-allergic Agents——Inhibition of the activation of hyaluronidase was determined by the above method after incubation at 37 °C for 20 min in acetate buffer of pH 3.5 containing the activator and hyaluronidase which had been preincubated with the anti-allergic agent at 37 °C for 20 min in the same buffer. Buffer solution was added in place of the anti-allergic agent as a control. The percentage inhibition was calculated as follows:

$$\text{inhibition } (\%) = \frac{\text{control OD}_{585} - \text{sample OD}_{585}}{\text{control OD}_{585}} \times 100$$

Materials——Hyaluronidase (from bovine testes) was purchased from Sigma Chemical Co., St. Louis; its specific activity was 500 NFunit/mg protein. Hyaluronic acid potassium salt was from Wako Pure Chemical Co., Osaka, and compound 48/80 was from Sigma Chemical Co. DSCG was kindly supplied by the pharmacology department of this university. Tranilast was synthesized in this laboratory according to the patent.[12)]

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