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KININ-FORMING AND DESTROYING ACTIVITIES OF SALIVA

BY

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Kininase activity is present in saliva obtained from the oral cavity but not in saliva obtained by cannulation of the parotid or submandibular ducts. This activity originates from squamous epithelial cells from the oral mucous membranes. Experiments with various inhibitors indicated that the kininase activity of squamous epithelial cells differs from that of plasma, and resembles that of erythrocytes. Kinin-forming activity is present both in the glandular secretion and in the squamous epithelial cells.

Both kinin-forming and destroying enzyme activities have been demonstrated in various fluids and tissues of human origin (Lewis, 1960; Trautschold & Rüdel, 1963). Saliva contains both enzyme activities (Werle & von Roden, 1936; Amundsen & Nustad, 1964). The present investigation was devised to determine the origin of these activities in the secretion, as earlier results had suggested that the kininase activity of saliva originated from squamous epithelial cells present (Amundsen & Nustad, 1964).

On the basis mainly of inhibitor studies, Erdös, Renfrew, Sloane & Wohler (1963) concluded that the kininase activity of plasma was different from that of erythrocytes. The action of such inhibitors on the kininase activity of saliva was therefore examined and compared with their action on the kininase activities of erythrocytes and plasma.

METHODS

Human saliva. This was collected in two ways, by cannulation of the parotid and the submandibular ducts, and by collection from the oral cavity.

Squamous epithelial cells. These were obtained in two ways:

(1) By gentle abrasion from the oral mucous membranes. The collected material was suspended in 0.9% saline and centrifuged at 1,400 *g* for 10 min. The sediment was washed five times in 0.9% saline and then suspended (0.07 g wet weight/ml.) in tris-buffer of *p*H 7.4. The suspension was exposed to ultrasonic treatment for 10 min at 20,000 cycles/sec with an MSE ultrasonic disintegrator, and then centrifuged at 1,400 *g* for 10 min. The kininase activity of the cell suspension was investigated before the ultrasonic treatment, and compared with that of the final supernatant fluid.

(2) By centrifugation at 1,400 *g* for 10 min of saliva collected from the oral cavity. The sediment was washed, suspended in buffer, exposed to ultrasonic treatment and centrifuged in the same way as the cells obtained by abrasion of the oral mucous membranes.

Human erythrocytes and plasma. These were obtained by centrifugation of citrated venous blood. The erythrocytes were suspended in 0.9% saline, centrifuged at 1,400 *g* for 10 min and washed five times in 0.9% saline. A suspension of washed erythrocytes in tris-buffer of *p*H 7.4 (1 g wet weight/ml.) was exposed to ultrasonic treatment for 10 min at 20,000 cycles/sec with an MSE ultrasonic disintegrator. The solution obtained was centrifuged and the supernatant fluid was diluted 1:6 with tris-buffer to a stock solution of *p*H 7.4. The citrated plasma was diluted 1:4 with tris-buffer to obtain a solution with a kininase activity quantitatively similar to that of the stock solution from disintegrated erythrocytes.

Bradykinin activity. This was determined on the uterus of virgin rats weighing 150 to 200 g. The rats had been brought into oestrus by intraperitoneal injection of 30 μg of stilboestrol 20 hr before removal of the uterus. The uterus was suspended in a 10 ml. organ bath containing de Jalon solution at 30° C. The time of contact was 1 min. Tests were made every 5 min.

Kininase activity. This was determined by incubation at 37° C of synthetic bradykinin (Sandoz) with the fluid sample to be investigated, and by taking aliquots after various time intervals for testing on the rat uterus. The initial concentration of added bradykinin was 0.5 μg/ml. in all incubates to be tested.

Inhibitors of kininase activity. The metal salts used (manganese sulphate, nickel sulphate, cobalt chloride and mercuric chloride) were of "pro analyse" quality and obtained from E. Merck AG, Darmstadt, Germany. *p*-Chloromercuribenzenesulphonic acid and 1,10-phenanthroline were obtained from Sigma Chemical Company, St. Louis, Missouri, U.S.A. Disodium edetate and $\alpha\alpha'$-dipyridyl were obtained from Fluka AG, Buchs, Switzerland. Potassium cyanide was obtained from the British Drug Houses, Ltd., Poole, England.

A stock solution was prepared of each inhibitor in tris-buffer, making the concentration of inhibitor five times that desired in the final test incubate. The *p*H of the solution was 7.4. Each inhibitor solution was first incubated for 30 min at 37° C with the fluid to be tested for kininase activity before the assay was started. The kininase activity of a fluid to which inhibitor had been added was compared with that of the same fluid equally diluted with tris-buffer.

Kinin-forming enzyme activity of squamous epithelial cells. Sediment obtained by centrifugation of saliva at 1,400 *g* for 10 min was washed five times in 0.9% saline and then suspended in tris-buffer (0.6 g wet weight/ml.). The suspension was exposed to ultrasonic treatment and centrifuged as previously described. The sediment was discarded, and the kinin-forming activity of the supernatant fluid was investigated before and after destruction of the kininase activity by acid treatment (Horton, 1959). One part of the supernatant fluid was incubated at 37° C with four parts of a stable substrate plasma (Amundsen, Nustad & Waaler, 1963), and the incubate tested for plasma kinin activity at intervals.

RESULTS

Origin of kininase activity of saliva

No kininase activity was found in saliva obtained by a smooth cannulation of the parotid or submandibular ducts (Fig. 1). The experiments of Fig. 1 show that although bradykinin was incubated with such saliva for as long as 30 min no inactivation of the peptide occurred. Moreover, suspensions of washed squamous epithelial cells from the oral mucous membranes showed considerable kininase activity when incubated with bradykinin under the same conditions. Further, ultrasonic disintegration of the suspended cells gave rise to an increase in this activity (Fig. 1). It was sometimes difficult to cannulate the submandibular duct without causing abrasion of cells from the duct wall. On these occasions, when cells could be demonstrated in the saliva, a small amount of kininase activity could be detected.

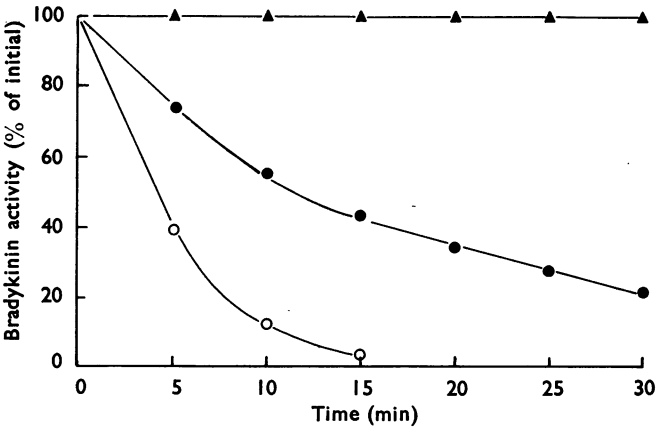

Fig. 1. Kininase activity of cell-free saliva and of suspensions of epithelial cells from oral mucous membranes. Incubation at 37° C was of 0.1 ml. of a solution of synthetic bradykinin (5 μg/ml.) with 0.9 ml. of saliva obtained by cannulation of a parotid duct (▲); and with 0.9 ml. of a suspension of washed epithelial cells before (●) and after (○) ultrasonic treatment of the cells at 20,000 cycles/sec for 10 min. 0.1 ml. volumes of the various incubates were tested on the rat isolated uterus preparation at intervals.

Effect of inhibitors on kininase activity

The effect of various inhibitors on the kininase activity of squamous epithelial cells is shown in Table 1. All the inhibitor studies were carried out using the same suspension of cells which was stored at −20° C in small portions until used. The kininase activity did not change during the storage period. The activity contained in 0.9 ml. of this fluid inactivated the standard amount of added bradykinin (0.5 μg) within 10 min. In order to facilitate a comparison of the effects of inhibitors on kininases from different sources, the plasma and the stock solution from haemolysed erythrocytes were diluted with tris-buffer of *p*H 7.4 so that these dilutions also inactivated the added bradykinin within 10 min.

The results shown in Table 1 show that the inhibitors manganese sulphate, nickel sulphate, cobalt chloride, zinc chloride, and *p*-chloromercuribenzenesulphonic acid distinguish the kininase activity of the squamous epithelial cells from that of plasma. With the exception of disodium edetate all the inhibitors studied had similar effects on the kininase activities of squamous epithelial cells and erythrocytes. In a concentration of 7.10^{-3}M, disodium edetate was a good inhibitor of the kininase activity of squamous epithelial cells, but a less potent inhibitor of the kininase activity of erythrocytes. An increase in the concentration of disodium edetate caused the kininase activity of erythrocytes to increase, whereas the kininase activity of squamous epithelial cells was further inhibited.

Zinc chloride was a good inhibitor of the kininase activity of squamous epithelial cells, and a less potent inhibitor of the kininase activity of erythrocytes. The inhibitor effect of zinc chloride on the kininase activity of squamous epithelial cells could, however, be reduced or overcome by addition of increasing amounts of disodium edetate to the incubates.

TABLE 1

EFFECT OF KININASE INHIBITORS ON THE KININASE ACTIVITIES OF HUMAN PLASMA, ERYTHROCYTES AND SQUAMOUS EPITHELIAL CELLS

A good inhibitor is marked + +. This implies a prolongation of the break-down period from 10 min to 30 min or more. The less potent inhibitors are marked + and the inactive ones 0. * indicates enhanced activity

Inhibitors	Concentration (M)	Relative degree of inhibition for: Squamous epithelial cells	Erythrocytes	Plasma
$MnSO_4$	3×10^{-4}	+	+	0
$NiSO_4$	3×10^{-4}	++	++	0
$CoCl_2$	3×10^{-3}	++	++	0
$ZnCl_2$	3×10^{-4}	++	+	0
p-Chloromercuribenzene-sulphonic acid	3×10^{-4}	+	++	0
$HgCl_2$	3×10^{-4}	++	++	+
KCN	3×10^{-3}	0	0	0
$\alpha\alpha'$-Dipyridyl	3×10^{-3}	++	++	+
1,10-Phenanthroline	3×10^{-4}	++	++	++
Disodium edetate	7×10^{-3}	++	+	++
Disodium edetate	$1{\cdot}4\times10^{-2}$	++	*	++

Kinin-forming enzyme activity of squamous epithelial cells

The supernatant fluid after centrifugation of ultrasonically treated suspensions of washed squamous epithelial cells showed no kinin-forming activity when incubated with the stable substrate plasma (Fig. 2). But the cells did contain some

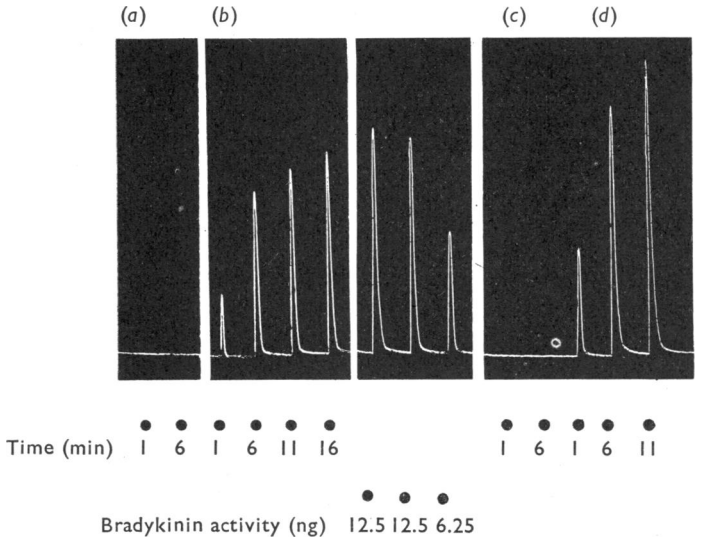

Fig. 2. Kinin-forming activity of ruptured squamous epithelial cells. Squamous epithelial cells from saliva were washed five times, suspended in tris-buffer of *p*H 7.4 (0.6 g wet weight/ml.), exposed to ultrasonic treatment for 10 min at 20,000 cycles/sec, centrifuged at 1,400 *g* for 15 min. and the sediment was discarded. Incubation was at 37° C of 0.2 ml. of test fluid with 0.8 ml. of substrate plasma. The record shows contractions of the rat isolated uterus preparation on addition of 0.1 ml. of incubates to a 10 ml. organ-bath. Test fluids: (*a*) 0.2 ml. of supernatant fluid from ultrasonically treated cells; (*b*) 0.2 ml. of the same supernatant fluid, after acid treatment; (*c*) 0.2 ml. of tris-buffer; and (*d*) 0.2 ml. of kininase-free saliva from the cannulated duct of a parotid gland.

kinin-forming enzyme activity, because when the kininase activity of the supernatant fluid had been destroyed by acid treatment, plasma kinin activity could be demonstrated in the incubate (Fig. 2). However, the squamous epithelial cells accounted only for a small part of the kinin-forming activity of saliva. The experiment of Fig. 2 shows that saliva collected from a cannulated duct contained a considerably greater amount of enzyme activity than that of a cell suspension. The difference depicted is accentuated by the fact that the 0.2 ml. of the cell-derived enzyme solution represented the total cell-derived kinin-forming capacity of approximately 8 ml. of saliva collected from the oral cavity.

DISCUSSION

This investigation has established that the kininase activity previously demonstrated in saliva originates from the squamous epithelial cells present.

The effect of inhibitors indicates that the kininase activity of squamous epithelial cells is different from that of plasma, but in many ways similar to that of erythrocytes. However, edetate inhibits the kininase activity of squamous epithelial cells, whereas in certain concentrations it enhances the kininase activity of erythrocytes. This may have some connexion with the different content of zinc in these cells; the erythrocytes contain much of the zinc-dependent carbonic anhydrase. The effect of addition of zinc chloride and edetate to the solution of disrupted squamous epithelial cells is in agreement with this supposition.

It has previously been demonstrated that human saliva contains plasma kinin-forming enzymes (Werle & von Roden, 1936). This investigation has demonstrated that such enzymes are present also in the squamous epithelial cells in saliva. Cell-free saliva obtained by cannulation of the parotid and submandibular ducts contains plasma kinin-forming enzymes but no kininase activity. This indicates that the kinin-forming capacity of saliva originates from the glands themselves.

Although the presence in squamous epithelial cells of enzymes that form as well as those that destroy plasma kinins does not prove that plasma kinins are related to or are important for the function of these cells, the possibility arises that these enzymes play a role in the activity of cells. It is interesting to speculate that the cells of other tissues may also contain these enzyme activities. We are at present investigating this possibility.

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