point to a decrease in the functional power of the mechanisms of ionic transport in the presence of massive hypertrophy of the heart [2, 3, 4, 9, 14]. This suggests that during adaptation to physical exercise, the power of the ionic transport system in heart muscle cells increases.

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EFFECT OF SALIVARY KALLIKREINS ON MICROVESSELS OF THE HAMSTER RETROBUCCAL POUCH AND RAT MESENTERY

R. D. Barabash, P. N. Aleksandrov, and S. V. Vovchuk

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The effect of purified human, rat, and hamster salivary kallikreins on microvessels of the rat mesentery and hamster retrobuccal pouch was studied by intravital microscopy. Dilatation of arterioles, a reduction in diameter of the venules, and an increase in the number of active capillaries were found. These effects differed depending on the species and organ concerned. Homogenates of the mesentery and retrobuccal pouch, proteins of the albumin type, histamine, and certain amino acids were found to activate the salivary kallikreins.

KEY WORDS: kallikrein; saliva; blood supply; digestive organs.

The physiological role of the salivary kallikreins in the digestive system has virtually not been studied except for their participation in the development of working hyperemia of the salivary glands [12].

The object of this investigation was to study the effect of purified salivary kallikreins on the microcirculation in the rat mesentery and hamster retrobuccal pouch.

EXPERIMENTAL METHOD

The effect of purified kallikreins isolated by the writers' own methods from rat, hamster, and human saliva [3, 5], on microvessels 10-75 μ in diameter in the mesentery and retrobuccal pouch was studied in ex-

Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. Research Institute of Stomatology, Ministry of Health of the Ukrainian SSR, Odessa.

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TABLE 1. Changes in Diameter of Microvessels (in % of initial) in Rat Mesentery and Hamster Retrobuccal Pouch under the Influence of Salivary Kallikreins (M ± m)

Preparations	Dose (in μg)	Rat mesentery		Hamster retrobucal pouch		
		venules (15-60 μ)	arterioles (10-50 μ)	venules (35-75 μ)	arterioles 45—75 (μ)	arterioles 20—40 (μ)
Salivary kallikreins:						
rat	4	—16,1±2,8	+21,5±6,4	—9,7±1,5	—10,0±2,3	+8,3±3,1
hamster	56	—16,2±5,1	+15,0±3,8	—10,0±4,0	—5,3±1,5	+7,7±3,1
human	170	—11,0±1,2	+10,0±4,1	—10,8±5,5	—10,8±0,8	
Bradykinin	0,5	—31,5±2,6	+13,3±4,2	—13,5±0,5	—9,0±1,7	

Legend. The dose given indicates the quantity of active substance in solution per application. +) Increase, −) decrease in diameter of microvessel.

experiments in vivo on 32 Wistar rats and 25 golden hamsters. Solutions were applied to the surface of the organs exteriorized into a thermostatically controlled chamber. The diameter of the microvessels was recorded instravitally by an image-splitting method [1]. In experiments in vitro the effect of rat and hamster blood serum, homogenates of the mesentery and retrobuccal pouch, bradykinin (Sandoz, Switzerland), histamine (Fluka, Switzerland), acetylcholine chloride (USSR), heparin (Gedeon Richter, Hungary), protamine sulfate (Spofa, Czechoslovakia), collagen, elastin, L-histidine hydrochloride, glycine (Reakhim, USSR), serotonin-creatinine sulfate, lyophilized human serum albumin, and L-arginine and L-hydroxyproline (Reanal, Hungary) on the activity of the salivary kallikreins was investigated. The concentration of the substances added and the doses of the kallikreins are given in Tables 1 and 2. The protein concentration in the eluates was determined by Lowry's method [15]. The concentrations and doses of the three isolated kallikreins were chosen so that their BAEE (N-α-benzoyl-L-arginine ethyl ester) -esterase activity [2] was the same. Kininase activity in the tissue homogenates was determined relative to hydrolysis of hippuryl-L-lysine [7]. The numerical results were subjected to statistical analysis [6].

EXPERIMENTAL RESULTS

The salivary kallikreins and bradykinin induced dilatation of the arterioles in the rat mesentery and reduced the diameter of the venules (Table 1), together with the appearance of peripheral stasis of the leukocytes in the venules, an increase in the number of open (active) capillaries, and acceleration of the lymph flow and an increase in the number of cells in the lymphatics. These phenomena are evidence of an increased intensity of capillary—tissue exchange in the mesentery under the influence of the salivary kallikreins [8]. Incidentally, dilatation of arterioles [13, 20], swelling of the endothelium of the venules [14], and an increase in the pressure in the venules [17] are characteristic responses of the mesenteric microvessels to bradykinin. Consequently, the salivary kallikreins act on the mesenteric microvessels not directly, but through kinin formation.

Since the kallikreins of rat saliva preserve their activity throughout the length of the digestive tract [4] and since experiments with kallikreins from the submandibular glands and pancreas have shown that they readily penetrate through the intestinal mucosa of rats into the mesenteric vessels [16, 19], it can be assumed that under natural conditions also the salivary kallikreins participate in the regulation of the blood supply of the digestive organs.

The response of the microvessels of the hamster retrobuccal pouch to the substances studied differed somewhat from the response of the mesenteric vessels described above. For instance, dilatation in response to rat and hamster salivary kallikreins developed only in arterioles of small diameter (20-40 μ). Arterioles of larger diameter responded by constriction to the application of all kallikreins and bradykinin (Table 1), possibly a result of species differences in the reactivity of the vessels of the retrobuccal pouch. This hypothesis is confirmed by observations showing that the sensitivity of the retrobuccal pouch microvessels to certain kinins is much weaker than that of the rat mesentery [10].

Investigation of the effect of the tissues of the two organs on kallikrein activity showed that homogenates of the bloodless mesentery and retrobuccal pouch caused substantial activation of salivary kallikreins (Table 2). No kininase was found in the tissue homogenates. Rat blood serum inhibitor exhibited narrow species-specificity toward salivary kallikreins, but hamster blood serum inhibitor inhibited all three kallikreins. In the search for individual substances activating salivary kallikreins in the homogenates, biologically active substances of the mast cells and certain proteins and free amino acids were tested. This choice was based on data showing the ability of collagen to adsorb kallikreins [11], marked activation of mouse salivary gland proteases by most amino acids [18], and the degranulation of mast cells participating in the regulation of the local blood flow [8]

TABLE 2. Changes in Kallikrein Activity in Vitro under the Influence of Certain Substances (M ± m)

Preparation	Dose (in μg/ml)	Homogenate of rat mesentery	Homogenate of hamster retrobuccal pouch	Rat blood serum	Hamster blood serum	Bradykinin	Histamine	Serotonin	Acetylcholine
		1 mg/ml		1:200		μg/ml			
Salivary kallikreins:									
rat	0,072	+36,7±8,8	0	−29,3±6,9	−63,0±10,1	−52,2±8,5	0	−41,6±7,6	0
hamster	1,36	+35,8±7,4	+54,8±16,3	+19,9±8,0	−54,2±12,4	−51,9±11,6	+19,8±4,0	−24,9±8,0	0
human	8,3	+31,9±4,7	+27,0±7,8	+51,2±2,6	−15,0±3,3	−54,2±4,5	+15,5±3,1	0	0

Preparation	Dose (in μg/ml)	Heparin 1000 units/ml	Albumin 250	Collagen 250	Elastin 250	Protamine 250	Arginine 200	Histidine 200	Glycine 200	Hydroxyproline 200
			μg/ml							
Salivary kallikreins:										
rat	0,072	−17,2±7,2	+15,8±9,7	0	0	−68,6±8,9	0	−14,7±3,1	0	0
hamster	1,36	−31,2±5,2	+50,7±11,0	0	0	−53,0±6,0	0	−26,4±3,8	0	0
human	8,3	−8,8±3,6	+37,1±4,0	0	+12,9±5,5	−82,2±6,6	+28,5±7,6	0	+13,0±4,1	+15,8±5,2

Legend. Mean values of increase (+) or decrease (−) in kallikrein activity expressed as percentages of activity of control samples containing only enzyme and physiological saline.

through the action of kallikrein [9]. Most of the substances tested either did not affect or inhibited (heparin, bradykinin, and the competitive substrate, protamine) the rate of BAEE hydrolysis, and only low-molecular-weight proteins of the albumin type significantly activated all three salivary kallikreins, histamine inactivated human and hamster kallikreins, and the free amino acids inhibited human kallikrein only. The activating action of rat blood serum on hamster and human kallikreins also was evidently linked with albumins (Table 2), for in the dilutions used, blood serum had virtually no BAEE-esterase activity.

The results thus indicate that the ability of salivary kallikreins to regulate the blood supply of the digestive organs possesses species specificity and is largely dependent on the properties of the organs at different levels of the digestive tract.

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