MORPHOMETRIC STUDY OF BLOOD CAPILLARIES FOLLOWING INJECTION OF FLUID INTO THE SALIVARY GLAND

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Physiological saline was injected into the efferent duct of the parotid salivary gland of cats under a pressure of 30, 70, and 120 cm water. It was shown by the use of transmission electron microscopy, morphometry, and statistical analysis that in response to injection of fluid into the gland the capillaries interweaving around the terminal secretory segments are compressed; compression of the capillary tubules is reflected in a marked decrease in the contribution of the working lumen of the capillaries and their endothelial lining to the total area; compression of the capillary tubules is accompanied by a reduction by half in the number of functioning capillaries. Two hypotheses are put forward on the basis of the results: 1) The additional volume of blood entering the gland in response to perfusion with fluid does not reach the blood capillaries but is discharged into the veins through communicating shunts; 2) regulation of the volume of blood entering the capillaries of the parotid salivary gland of the cat must depend on the hydraulic and osmotic conditions in the interstitial space of the gland lobules.

KEY WORDS: parotid salivary gland; functional hyperemia; blood capillaries; electron microscopy; morphometric analysis.

Working (functional) hyperemia in the salivary glands is most marked in the salivation phase [3, 4]. Injection of fluid into the duct of the gland has recently been used as an experimental model of working hyperemia [1, 5, 6]. If this method really reproduces a state of functional hyperemia, it can rightly be expected that the resistance to the flow of blood transported through the capillaries would be reduced. This conclusion can be drawn from the data of Haggendal and Sivertsson [7], who showed that all the additional volume of blood in the secreting salivary gland enters the capillaries and none passes through communicating shunts.

The object of this investigation was to study the character of geometric modifications to the capillaries after injection of fluid into the duct of the parotid salivary gland.

EXPERIMENTAL METHOD

Experiments were carried out on 23 cats. Physiological saline was injected, in accordance with Andronov's recommendations [1], into the dissected duct of the parotid salivary gland under a pressure of 30, 70, and 120 cm water. The salivary gland was fixed in situ with a 2.5% solution of glutaraldehyde in phosphate buffer, pH 7.4, 5 min after the beginning of perfusion. The gland was cut into blocks measuring 0.5-1 mm^3, which were postfixed in the same solution, washed in 0.1M phosphate buffer, and then immersed in osmium fixative [8] for 1.5-2 h. After dehydration, pieces of the gland were embedded in Araldite. A parotid salivary gland into whose efferent duct a cannula was introduced, but no physiological saline was injected, served as the control. Ultrathin sections were examined and photographed in the Hitachi Hs-9 electron microscope. The total area of capillary cross sections, the area of the working lumen of the capillaries, the area of their endothelial lining, and the area of the endotheliocyte nuclei were measured on survey photographs of sections through the capillaries. The total number of capillaries and also the number of capillary cross sections containing erythrocytes also were counted. The obtaining and processing of the quantitative information were in accordance with the algorithm of morphometric analysis suggested previously [2]. In the analysis of the data it was noted that segments of the capillary tubules containing nuclei differed from those without nuclei. The quantitative data were therefore analyzed separately.

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TABLE 1. Quantitative Parameters of Changes in Periacinar Capillaries of Cat Parotid Salivary Gland After Injection of Fluid Into Duct (M ± m)

Experimental conditions	Number of animals	Cross sections of blood capillaries containing nuclei				Cross sections of capillaries without nuclei		
		area of lumen of capillary, μ^2	area of endothelial lining of capillary, μ^2	area of nucleus of endotheliocytes, μ^2	area of cross section of capillary, μ^2	area of lumen of capillary, μ^2	area of endothelial lining of capillary, μ^2	area of cross section of capillary, μ^2
Control	6	12,3±1,51 (25)	7,99±0,78 (25)	6,58±0,48 (25)	26,83±2,04 (25)	12,36±1,81 (25)	4,88±0,47 (25)	17,52±2,10 (25)
Injection of physiological saline under pressure of:								
30 cm water	5	3,94±0,95* (26) 68%	4,80±0,59 † (26) 40%	4,47±0,55 (26) 32%	13,20±1,36* (26) 50,8%	4,48±0,64* (28) 64%	4,43±0,39 (28) 9,2%	8,90±0,84* (28) 49,2%
70 » »	7	6,35±1,11† (27) 48,4%	5,22±0,50 † (27) 34,8%	5,24±0,65 (27) 20,3%	16,84±1,64* (27) 37,2%	7,60±0,62 ‡ (38) 38,5%	4,65±0,41 (38) 4,5%	12,25±0,62 ‡ (38) 30%
120 » »	5	3,38±0,86* (25) 72,6%	5,73±0,56 ‡ (25) 28,3%	7,84±0,76 (25) 16%	16,94±1,20* (25) 37%	4,29±0,56* (45) 65,3%	5,78±0,45 (45) 16%	10,06±0,67* (45) 42,6%

Legend. 1. Size of sample given in parentheses. 2. Change in parameter relative to control expressed in per cent. 3. *) $P < 0.001$, †) $P < 0.01$, and ‡) $P < 0.025$ compared with control.

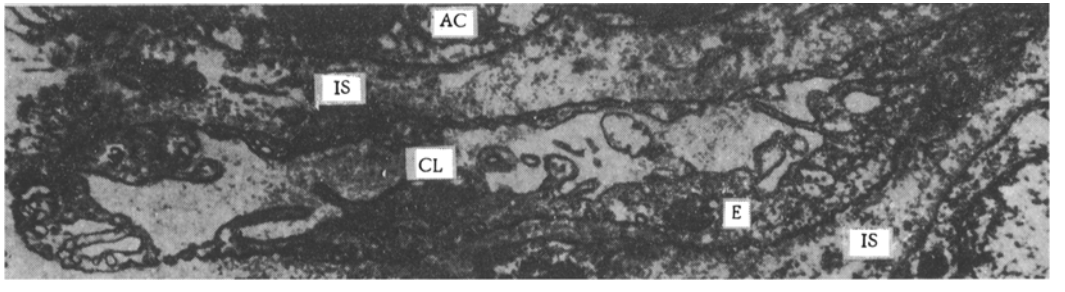

Fig. 1. Elliptical shape of cross section of blood capillary from cat parotid salivary gland after injection of physiological saline into efferent duct under pressure of 70 cm water. CL) Capillary lumen; E) endothelium; IS) interstitial space; AC) acinar cell. 21,000×.

EXPERIMENTAL RESULTS

Visual comparison of the shape of the cross section of the capillaries in the control and experimental animals showed that injection of fluid into the gland was followed by an increase in the number of capillary cross-sections discovered whose shape resembled that of a compressed ellipse (Fig. 1). These observations suggested that changes in the fluid balance in the tissues of the gland lead to geometric modifications to the capillary tubes.

From the morphometric data and the results of the calculations based on them, the following conclusions were drawn (Table 1): 1) In response to injection of fluid into the gland, the blood capillaries interweaving around the terminal secretory segments are compressed; 2) compression of the capillary tubules is expressed as a marked decrease in the contribution not only of the area of their working lumen (by 63% for the nucleated segments of the capillaries and by 55.9% for those without nuclei), but also the area of the endothelial lining (by 34.4% for the nucleated segments of the tube) to the total area; 3) an increase in the perfusion pressure of the fluid above 30 cm water does not lead to any statistically significant increase in compression of the periacinar capillaries.

Analysis of these results and of data in the literature [5, 6, 9, 10] suggests that injection of physiological saline into the salivary gland leads to hydration (swelling) of the interstitial gel, elevation of the hydrostatic pressure of the interstitial fluid (swelling pressure [11]) and, consequently, to collapse of the capillary tubes. With this possibility in mind, changes in the number of erythrocytes in the capillary lumen were analyzed. Statistical analysis showed that in the control series erythrocytes were present in 38.3% of the total number of cross sections of the periacinar capillaries of the cat parotid salivary gland examined. Injection of physiological saline into the duct of the salivary gland under different pressures led to a decrease in the number of cross sections of the capillaries containing erythrocytes. For instance, under a pressure of 30 cm water erythrocytes were found in 18.18% of the total number of cross sections of capillaries examined, under a pressure of 70 cm water in 18.75%, and under the pressure of 120 cm water in 16.7%.

The results of statistical analysis thus suggest that the number of functioning capillaries in the cat parotid salivary gland was reduced by half by injection of fluid into its efferent duct. The decrease in the number of functioning capillaries means an increase in the resistance to the flow of blood along the capillary tubes.

Consequently, two hypotheses can be put forward: 1) the additional volume of blood entering the gland in response to its perfusion with fluid [1, 5, 6] does not reach the blood capillaries but is discharged into the veins via communicating shunts; 2) regulation of the volume of blood reaching the capillaries of the parotid salivary gland must depend on the hydraulic and osmotic conditions in the interstitial space of the gland lobules.

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