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PARAMETERS OF METABOLISM OF HEXOSAMINE-CONTAINING BIOPOLYMERS IN EXPERIMENTAL TOOTH REIMPLANTATION

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KEY WORDS: hexosamine-containing biopolymers; reimplantation of teeth

Renewed interest has recently been shown in a surgical method of treatment of chronic periodontitis, namely the reimplantation of teeth [1, 6]. However, the mechanisms of wound healing under these circumstances, and changes in metabolism of connective-tissue biopolymers, including hexosamine-containing biopolymers (HCB), glycosaminoglycans (GAG), and glycoproteins, have still received only little study. Accordingly it was decided to study parameters of HSB metabolism in the periodontal tissues and blood during the course of healing of uninfected wounds associated with one-stage reimplantation of teeth.

EXPERIMENTAL METHOD

Experiments were carried out on 16 male mongrel dogs aged 2-3 years. After preliminary examination by a veterinary surgeon the teeth were removed by the most sparing method under thiopental anesthesia. Four teeth were immediately reimplanted, namely the lateral incisor on both sides of each jaw. Neither curettage of the alveolus nor endodontic treatment of the teeth was carried out. After extraction of the teeth the alveoli were covered with sterile gauze swabs. The extraalveolar period amounted to 15-18 min. During this time the teeth were kept in physiological saline (18-22°C) with penicillin (100,000 U/ml) and streptomycin (100 mg/ml), and with observance of the rules of asepsis. After reimplantation of the teeth in the appropriate alveoli, they were fixed to neighboring teeth by means of quick-hardening plastic.

Of the 16 experimental animals, normal healing of all the reimplanted teeth took place in 14 animals (87.5%), and these were further investigated.

After 14 (Group 1, seven dogs) and 28 (Group 2, seven dogs) days of observation the animals were killed under short-term ether anesthesia. Fragments of the upper and lower jaws were cut with a saw to include experimental (reimplanted) and intact (control, next to the reimplanted) teeth. The fragments of the jaws were freed from soft tissues mechanically, and the roots of the teeth, tissues of the periodontium, and bony tissue of the alveoli were isolated. The total content of HCB [3, 4] and GAG [5] in these tissues was determined and expressed in millimoles hexosamines and hexuronic acids per kilogram dry weight of defatted tissue respectively. Hexosamine synthetase activity (HSA) was determined in the supernatant (3000 rpm, 10 min) of periodontal tissue homogenate and expressed in nanomoles/mg protein/h [7]. Every 7 days during healing, the GAG level was determined in citrated plasma from blood of both groups of animals [5].

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TABLE 1. Parameters of HCB Metabolism During Reimplantation of Teeth (M ± m)

Tissues studied	Hexuronic acids, mmoles/kg	Hexosamines, mmoles/kg	HCB, nmoles/mg/h
Root of tooth, control	3,10±0,01	7,23±0,04	
Reimplantation:			
After 14 days	3,49±0,09	7,72±0,08	
After 28 days	3,31±0,07	7,53±0,07	
Periodontium of tooth, control	7,82±0,03	16,62±0,08	3,95±0,07
Reimplantation			
After 14 days	9,61±0,10*	22,90±0,14*	8,84±0,11*
After 28 days	8,21±0,11	18,50±0,12	4,56±0,09
Bone tissue of alveolus, control	4,61±0,02	9,08±0,06	
Reimplantation			
After 14 days	4,97±0,07	11,34±0,11*	
After 28 days	4,82±0,06	10,03±0,09	

Legend. *$p < 0.05$.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that the GAG content in the root of the teeth, periodontium, and bony tissue of the dental alveolus of the intact animals was 3.10, 7.82, and 4.61 mmoles hexuronic acids/kg dry weight of defatted tissue respectively. Our data on the GAG level in the root of the tooth and bone tissue differ only a little from those in the literature [3, 8].

During one-stage reimplantation of teeth in the upper and lower jaws of the dogs the GAG content in the periodontium rose after 14 days by 22.8%, but after 28 days it was back to normal. This parameter underwent no significant changes in the root of the teeth or the bony tissue of the alveoli of the reimplanted teeth.

The GAG content, measured as level of hexuronic acids, in the blood plasma of the dogs before the operation (control) was 42.3 ± 2.1 μmoles/liter. Their content 7, 14, 21, and 28 days after reimplantation was 48.3 ± 4.6 (+11.4%), 44.2 ± 3.4, 46.7 ± 2.8, and 41.9 ± 2.7 μmoles/liter respectively. These changes were not significant.

The HCB level, determined as hexosamines, in the root of the tooth 14 and 28 days after the operation was virtually unchanged. An increase in the content of the test biopolymers in the periodontium and bony tissue of the alveolus of the experimental teeth was increased by 37.7 and 24.8% respectively on the 14th day of the experiment. By the end of the experiment the HCB content in these tissues did not differ significantly from normal.

HCB are known to consist of GAG, glycoproteins, and hexosamine-containing glycolipids. An increase in their total level in the test tissues may take place on account both of migration of plasma glycoproteins and of an increased rate of their local biosynthesis. However, a parallel increase in the GAG content and in the rate of biosynthesis of hexosamines (precursors in HCB formation) in the periodontium by the 14th day of the experiment is evidence that local synthetic processes make the greatest contribution to the increase in HCB [2].

During reimplantation of teeth, processes of resorption, regeneration, and calcification, demonstrated morphologically, are characteristic of healing of fractures, with definite changes in metabolism of connective-tissue biopolymers, including HCB [2-4]. Judging by the very low percentage (12.5) of unsuccessful outcomes, a very small change in blood levels of HC metabolism and definite normalization of the parameters of HCB metabolism in the periodontal tissues by the 28th day of the experiment, indicate that one-stage noninfected reimplantation of teeth is a relatively nontraumatic operation. The results also justify existing stomatologic practice, namely early functional use of the reimplanted teeth.

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SODIUM–CALCIUM EXCHANGE IN SMALL INTESTINAL MYOCYTES

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One way of removing Ca^{2+} ions from the smooth-muscle cell is Na/Ca exchange, carried out by means of membrane carriers. This process has been sufficiently well studied on plasma membranes (PM) of skeletal and heart muscles, nerve fibers, blood cells, etc. Information on this type of ion antiport in smooth muscles is very restricted, and results obtained on the myometrium [2] points to its electrical neutrality.

In this paper we describe experimental proof of the substrate saturability of the transfer, its specificity for Na^+ and Ca^{2+}, and the reversibility and electrogenicity of the antiport.

EXPERIMENTAL METHOD

Experiments were carried out on the vesiculated fraction of PM of rabbit small intestinal myocytes [6], using $^{45}Ca^{2+}$ and an "Orion 93-20" Ca-selective electrode. The concentration of PM protein was 100 μg/ml when exchange was investigated by means of an electrode and 1-5 μg/ml when the isotope method was used.

EXPERIMENTAL RESULTS

Investigations showed that membrane vesicles containing NaCl, when added to sodium-free medium with 30 μM $CaCl_2$, can accumulate calcium. Na-dependent Ca^{2+} accumulation by vesicles depends on the intravesicular Na^+ concentration (Fig. 1, curve 1), and is evidence that the motive force of the antiport is the sodium gradient. With reduction of this gradient calcium accumulation by membrane vesicles falls (Fig. 1, curve 2) to the level corresponding to the quantity of cation bound by the membranes [4]. Conversely, a similar increase in K^+ in the medium does not affect accumulation of Ca^{2+} by vesicles, but in the presence of valinomycin, accumulation is increased (Fig. 1, curve 3).

The results can be explained as follows. Under conditions when the incubation medium contained KCl and valinomycin, the membrane voltage with "+" sign was created inside the vesicles, for initially the vesicles did not contain K^+ ions. Thus a positive charge inside the membrane vesicles stimulates Na/Ca exchange. The maximal velocity of the process is increased under these circumstances, but affinity for the Ca^{2+}-system of the antiport is unchanged: K_m for Ca^{2+}, calculated from data shown in Fig. 2, in both the presence and the absence of valinomycin, was 20 μM.

Comparison of the quantity of Ca^{2+} accumulated by membrane vesicles with the quantity of Na^+ escaping from the vesicles into the medium containing sucrose (or KCl) and KCl + 3 μM valinomycin, showed the following relationships. Within the range of extravesicular Ca^{2+} concentration up to 40-50 μM (saturation concentration of the antiport), this ratio between the cations varied from 1.9 to 2.25 in medium without valinomycin and from 2.27 to 3.12 in medium with the ionophore. In the first case the ratio of Ca^{2+} entering the vesicles and sodium leaving them corresponded to the $1Ca^{2+}$:$2Na^+$ stoichometry (nonelectrogenic antiport), $1Ca^{2+}$:$3Na^+$ in the second (electrogenic mode of operation of Na/Ca exchange).

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