INVESTIGATION OF THE PROPERTIES OF A HYALURONIDASE PREPARATION ISOLATED FROM THE FEMALE REPRODUCTIVE SYSTEM

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At a certain period of the sex cycle an enzyme with hyaluronidase action is secreted by the female reproductive system (oviducts and uterus); its activity is highest during the period of estrus. The possible role of the hyaluronidase of the oviduct in the physiology of fertilization is postulated. A new source for obtaining hyaluronidase preparations, the uterine mucosa of sexually mature animals, is suggested. These preparations have specific activity and are nontoxic.

KEY WORDS: hyaluronidase; uterolidase; uterine mucosa.

Numerous investigations have demonstrated the participation of the biological system of hyaluronic acid–hyaluronidase in many aspects of physiological processes such as fertilization [2, 3, 10, 11, 16], the course of pregnancy and parturition [5, 13], and phenomena of growth, permeability, and regeneration [1, 13, 18, 19].

As well as the definite role of hyaluronidase in vitally important functions of the body, in recent years the important biological role of this enzyme in the development of certain pathological states connected with injury to the connective tissue has been established (complicated healing of fractures, pathological scars formation in the skin after thermal burns, etc. [6, 7]).

The great importance of the current extensive use of preparations with hyaluronidase action (lidase, ronidase) in medical practice at the present time for the treatment of inflammatory diseases of the female reproductive system and of sterility, and to accelerate the absorption of therapeutic substances, the revolution of scars, and the healing of wounds [3, 12], will be evident on the basis of the facts described above.

The chief sources of this enzyme at present are the testes of sexually mature cattle [4, 15] and certain strains of microorganisms [8, 14]. There are reports [8, 14] that preparations of hyaluronidase obtained from different sources may differ in some of their physicochemical properties and that they may possess marked antigenic activity and high serological specificity, as a result of which the use of any single hyaluronidase preparation for therapeutic purposes must reduce its effectiveness.

In view of these facts the need for further search for fresh sources of enzymes with hyaluronidase action will be understood. The fact that at certain periods of the sex cycle hyaluronidase is secreted in the oviducts and uterus is interesting in this connection [10, 11]. Joint investigations with E. T. Mikhailenko showed that an enzyme with hyaluronidase action is also present in the human oviducts. The uterus of animals also contains a certain quantity of hyaluronidase [11], sufficient for the enzyme to be isolated preparatively.

This paper describes the results of comprehensive investigations of certain properties of a hyaluronidase preparation obtained from a fresh source (the uterus) under laboratory and production conditions.

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EXPERIMENTAL METHOD

A preparation of hyaluronidase called uterolidase was obtained from the uterus of animals, at first under laboratory and later under production conditions, at the Medical Preparations Department of the L'vov Meat Combine, by the method used for the production of testicular lidase [11, 15]. The uterolidase was tested for toxicity and specific activity in the laboratory of the Medical Preparations Department and for total proteolytic activity, using casein as the substrate, by Kunitz's method in the biochemistry laboratory (Head, Professor K. N. Veremeenko) of the Kiev Scientific-Research Institute of Otolaryngology.

The preparation was investigated by chemical [17, 20], viscometric [9], and biological methods. Experiments were carried out on 20 rabbits in 2 variants. In the first variant 0.25 ml of a mixture of the preparation with 2% trypan blue solution (indicator) in equal proportions was injected intradermally. The area of diffusion of the indicator was measured 5 min and again 20 h later. The final area of diffusion was divided by the initial area of the stain and the result gave the coefficient of spread; if the area of the stain in the experimental series (preparation with indicator) was divided by the area in the control (Physiological saline + indicator) the result gave the index of diffusion (Claude's index). In the second variant the biological tests were carried out as follows: the preparations for testing was injected intradermally and this was followed immediately by intravenous injection of the indicator in physiological saline. The time of appearance of the color at the site of intradermal injection of the test preparation was then determined. In these two variants of the biological tests, the Hungarian preparation of testicular hyaluronidase (300 USP units) also was used for comparison.

The hyaluronidase activity in 15 specimens of the uterolidase preparation was determined colorimetrically [17] in 0.1 ml of the preparation after the contents of the flask had been dissolved in 1 ml physiological saline. Activity of the enzyme preparation was judged from the quantity of N-acetylglucosamine (in μg) liberated during incubation for 18 h at 37°C in 0.1 M acetate buffer containing 0.15 mole/liter sodium chloride, pH 3.7. Hyaluronic acid (sodium salt; Reanal, Hungary) in a concentration of 4 mg/ml was used as the substrate.

The effect of uterolidase on the corona radiata and follicular cells was studied in vitro by incubation of oocytes extracted from the follicles before the beginning of ovulation in pigs in a solution of this enzyme. Oocytes also were incubated in a solution with hyaluronidase isolated from the oviducts.

EXPERIMENTAL RESULTS

The hyaluronidase preparation isolated from the uterus and lyophilized consisted of a white or pale-yellow mass, readily soluble in water, in 0.9% sodium chloride, and in glucose solutions. The highest activity of the enzyme (30-60 conventional units) was observed during estrus and it fell sharply or was completely undetectable during diestrus. Activity of the hyaluronidase isolated in these experiments [11] appeared in the same titer as that of preparations of testicular hyaluronidase of Soviet and Hungarian manufacture (1:32-1:128). The fluctuations in enzyme activity depended on the period of the sex cycle at which the uterus was taken as raw material. Studies of the other properties of the preparation showed that heparin (2200-4000 i. u. in 2 ml incubation mixture) and trypsin (0.4 mg to 1.5 ml mixture) completely inhibited 50-100 conventional units of the enzyme. Chemical tests of the uterolidase preparations also showed that they contained hyaluronidase activity, ranging from 22 to 38 μg of liberated N-acetylglucosamine.

In biological tests on rabbits (intradermal injection of the preparation with indicator) it was confirmed that uterolidase possesses high activity. Whereas 5 min after injection of the preparation the area of the stain was 19 cm^2, after 20 h this area increased to 155.9 cm^2, i.e., the coefficient of spread was 8.22 ± 0.86 ($P < 0.001$). In the control the stain during this period increased in area from 15.7 to 21.3 cm^2 (coefficient of spread 1.35 ± 0.05). The diffusion index in the experimental series averaged 7.3 ($P < 0.001$). Biological tests carried out by the second method (intradermal injection of the preparation, intravenous injection of the indicator) also showed that intestive staining of the zones took place at the sites of intradermal injection of both uterolidase and of bovine testicular lidase 10-20 min after the injection of trypan blue into a vein of the rabbit's ear, whereas in the control no staining appeared whatsoever. The results thus indicate that at the sites of injection of uterolidase the permeability of the vessels and tissues were increased, a characteristic feature of the action of the enzyme hyaluronidase. Tests for the presence of proteinases showed that uterolidase, like testicular lidase, has no proteolytic activity.

Tests in vitro of the action of hyaluronidase isolated from the uterus and oviducts on the corona radiata and groups of follicular cells surrounding the oocyte showed that under the influence of uterolidase

and of hyaluronidase isolated from the oviducts the firm corona radiata and its sorrounding cells underwent certain changes. After incubation the ground substance of the granulosa cells lost their elasticity and became viable. If drawn up into a fine glass pipet and carefully expelled and the procedure repeated several times, the oocytes, corona radiata, and follicular cells lost most of their mass. Of 30 oocytes incubated with hyaluronidase from the oviducts, for instance, in 21 oocytes the granulosa cells were scattered. Of 35 oocytes incubated with uterolidase, in 31 the corona radiata and groups of follicular cells were easily detached. In control specimens (incubation of the ova in physiological saline without the addition of hyaluronidase preparations) no significant changes took place in the corona radiata or follicular cells.

The experiments thus showed that at a certain period of the sex cycle an enzyme with hyaluronidase action is secreted by the female reproductive system and that, under the influence of this enzyme, the oocyte is prepared for the processes of fertilization. It can be postulated on the basis of these results that the hyaluronidase of the oviduct plays a role in the physiology of fertilization. The study of the properties of the enzyme preparations obtained from the oocytes and uterus of animals showed their specific activity and their nontoxicity. Hence it follows that the mucosa of the female reproductive tract can be used as a source for the production of hyaluronidase preparations. Considering that hyaluronidase preparations isolated from different sources (testicles, streptococci)are not identical as proteins and that they possess specificity as antigens [8, 14], by using this new source to obtain the enzyme the choice of hyaluronidase preparations can be widened and their therapeutic effectiveness enhanced.

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