HISTOCHEMICAL STUDY OF MASTITIC MAMMARY GLAND IN LACTATING COWS*

HISTOCHEMICKÉ ŠTÚDIUM ZÁPALOVO ZMENENEJ MLIEČNEJ ŽLAZY LAKTUJÚCICH DOJNÍC

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ABSTRACT: The activity of alkaline phosphatase, adenosine triphosphatase, acid phosphatase, and succinate dehydrogenase in the epithelial cells of the chronic, inflammatory, mammary gland in the lactating cows was determined by a densitometric analysis. In comparison with the intact mammary gland, the inflammatory disease led to expressive changes in all the enzyme activities. Significantly increased activity (p < 0.001) of alkaline phosphatase, adenosine triphosphatase, acid phosphatase, and succinate dehydrogenase was detected in the epithelial cells. The results of the experiment refer to a narrow relationship between the enzymatic activity and inflammatory process in the mammary gland during the lactation period.

alkaline phosphatase; adenosine triphosphatase; acid phosphatase; succinate dehydrogenase; histoencezymatic study; mammary gland; cows

ABSTRAKT: Densitometrickou analýzou bola stanovená aktivity alcalickej fosfatáz, adenosintrifosfatáz, kyslých fosfatáz a sukcinátehydrogenázy v epitelových bunkách mliečnej žlaz dojnic s chronickým zápalom mliečnej žlaz. Pri porovnaní s intaktnou mliečnou žlazou, zápalové ochorenie vedlo k signifikantnému zvýšeniu aktivity (p < 0.001) všetkých sledovaných enzýmov v epitelových bunkách. Výsledky experimentu poukazujú na úzky vzťah medzi enzymickou aktivitou a zápalovým procesom mliečnej žlaz počas laktácie.

alkalická fosfatáz; adenosintrifosfatáz; kyslá fosfatáz; sukcinátehydrogenáza; histochemické štúdium; mliečna žlaz; dojnice

INTRODUCTION

Nowadays, mastitis is still the most relevant and most costly infectious disease of dairy cows. The principal microorganisms (Streptococcus agalactiae, Staphylococcus aureus, Streptococcus uberis, Streptococcus dysgalactiae, Escherichia coli) cause expressive clinical and morphological changes (Philpot and Pankey, 1978) which can be determined biochemically and histochemically. Up to the present, research has been directed at the detection of clinical and morphological changes in the inflamed mammary gland (Vasil, 1994). Still, there is little data about the influence of infection on the enzyme activity in the cells of mammary gland. For the possibility of using a histochemical method, the work of Lenhardt et al. (1994) should be consulted. In this paper the alkaline phosphatase (AP), adenosine triphosphatase (ATP-ase), acid phosphatase (AcP), and succinate dehydrogenase (SDH) activities were detected in the healthy mammary glands of lactating cows.

The objective of this study was to determine the influence of chronic mastitis on the activity of alkaline phosphatase, adenosine triphosphatase, acid phosphatase, and succinate dehydrogenase in the parenchyma of mammary gland.

MATERIAL AND METHODS

Sixty lactating cows slaughtered at the slaughterhouses in Košice were used in the experiment. Material for histochemical evaluation was obtained from the parenchyma of 25 cows with clinically healthy mammary glands and from 35 cows with mastitis, with positive findings in bacteriological examination (Staphylococcus aureus, Streptococcus dysgalactiae, and Streptococcus uberis) detected in the parenchyma of mammary gland.

Samples sized 1 x 1 x 0.5 cm, were taken from the pars glandularis sinus lactiferis of the mammary gland.

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Samples were taken within 10 minutes of slaughter, frozen, and kept in a liquid nitrogen until processed. On the day of experiment, a segment of the frozen tissue was cut (7 μm) in the cryostat at −21 °C and the tissue slices were transferred to glass slides and air-dried. From each tissue segment, seven sections were cut for different enzyme assays.

Demonstration of alkaline phosphatase activity was performed by using a modified simultaneous azo-coupling method (Lojda et al., 1979). The incubation medium contained naphthol-AS-MX-phosphate (Fluka, Germany), and stable diazonium salt Fast Blue BB (Sigma, USA), veronal acetate buffer (pH 9.2). The incubation was performed at 37 °C for 30 min.

Adenosine triphosphatase activity was demonstrated by the lead salt method according to Lojda et al. (1979). The incubation medium contained adenosine triphosphate-sodium salt (Sigma, Germany), lead nitrate and magnesium sulphate (Aldrich, Germany), tris-maleate buffer (pH 7.2). The sections were incubated for 20 min at 37 °C.

Determination of acid phosphatase activity was performed according to Lojda et al. (1979). The incubation medium contained naphthol-AS-BI-phosphate (Fluka, Germany), hexazotized new fuchsins (Serva, Germany), veronal acetate buffer (pH 6.0). The incubation lasted for 10 hours at 37 °C.

Succinate dehydrogenase activity was performed according to Lojda et al. (1979). The medium consisted of succinate-disodium salt (Fluka, Germany), nitro BT, tris-HCl buffer (pH 7.2). The incubation lasted for 30 min at 37 °C. After incubation, sections were washed with distilled water to remove the incubation medium and to stop any reactions. Postfixation of the sections was performed in a solution of 4% (v/v) formaldehyde for 10 h at 20 °C. The sections were rinsed in distilled water and mounted in glycerin jelly.

Enzyme activity of the epithelial cells was cytophotometrically analysed with a Vickers-M-86 microdensitometer. The measurements were performed using a x 40 objective and effective scanning area of 28.3 μm² and scanning spot 0.5 μm. The integrating absorbance was measured at a wavelength of 480 nm for alkaline phosphatase and succinate dehydrogenase, 590 nm for adenosine triphosphatase, 520 nm for acid phosphatase.

The enzyme activity was evaluated as the absorbance values recorded by the instrument/min/μm² epithelial cells ± SD. The density of enzymes investigated was determined in six sections of each sample at 10 sites corresponding to the epithelial cells in the mammary gland. Statistical analyses were done by t-test.

RESULTS

Tab. I summarizes the measurements of enzyme activities in the epithelial cells of healthy and diseased mammary glands of lactating cows. In comparison with intact mammary glands, mastitis led to a significant increase in the activity of all the enzymes investigated.

I. Densitometric analysis of alkaline phosphatase, adenosine triphosphatase, acid phosphatase, and succinate dehydrogenase in the epithelial cells of healthy and diseased bovine mammary glands

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Bovine mammary gland</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>healthy (x ± SD)</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>15.66 ± 1.14</td>
</tr>
<tr>
<td>Adenosine triphosphate</td>
<td>15.70 ± 1.02</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>23.27 ± 1.85</td>
</tr>
<tr>
<td>Succinate dehydrogenase</td>
<td>12.10 ± 0.94</td>
</tr>
</tbody>
</table>

*significantly different from control p < 0.001

1. A histochemical picture of uniformly metachromatic activity of alkaline phosphatase in the epithelial cells, lower activity in myoepithelial cells and in the endothelial cells of the capillaries in mastitic mammary gland sections of lactating cows (obj. 6.3; eyepiece 6.3)
ranging from 1.0 (AcP) to 3.0 times (SDH) higher than with intact glands.

Differences in the enzyme activities in other structures of the mammary gland (myoepithelial cells and endothelial cells of the capillaries) are presented in Figs. 1–4.

Against the very noticeable activity of alkaline phosphatase in the epithelial cells, the activity of this enzyme was lower in the other structures investigated (Fig. 1).

Fig. 2 shows the presence of the reaction product of the membrane adenosine triphosphatase in the epithelial cells. Similarly, the vigorous activity of this enzyme was observed in the myoepithelial cells, and in the endothelial cells of capillaries.

Epithelial cells showed a marked activity of acid phosphatase. The reaction product of this enzyme was also observed in the myoepithelial cells; hardly any final reaction was found in the endothelial cells of capillaries (Fig. 3).

When tissue slices were incubated to demonstrate succinate dehydrogenase, an increased amount of the reaction product was detected in the epithelial cells in comparison with myoepithelial and endothelial cells (Fig. 4).

**DISCUSSION**

The results of our experiment showed the close relationship between enzyme activities and chronic mastitis in the cow’s mammary gland during lactation. Alkaline phosphatase is found primarily in the cell membranes of the cow’s mammary gland, where the active transport processes take place. In agreement with this, we detected the presence of AP in all the structures.
investigated both in the mastitic mammary gland as well as in the healthy udder (Lenhardt et al., 1994). Similar results were obtained by Korfmeier (1976): he detected positive reactions to alkaline phosphatase in the epithelial cells of the mammary gland in mice. He related the level of this enzyme activity to the coherent functioning of the mammary gland. The higher levels of alkaline phosphatase detected in our experiment during mastitis suggest a changed dysfunctioning of the mammary gland. Similarly, an increased activity of alkaline phosphatase in the proliferated fibroblasts around carcinomas was observed by Pachdaman and Stain (1963).

Cell membrane adenosine triphosphatase is responsible for the physiological degradation of adenosine triphosphate and takes place in the transport of potassium and sodium across membranes. In healthy cows the enzymatic activity of membrane-bound ATP-ase was high in the epithelial cells (Tab. 1). In agreement with this Korfmeier (1976) described a marked activity of ATP-ase in all structures of murine mammary gland investigated during the lactation period. Like in the case of AP, mastitis led to a noticeable increase in the ATP-ase activity in the investigated structures.

In the mammary gland of healthy cows, the acid phosphatase showed a marked activity in the epithelial cells and a moderate activity in the myoepithelial cells. Hardly any final reaction product was found in the endothelial cells of capillaries. Similar results were obtained by Michel (1979b), Longauer and Bilčík (1980, 1993), Lenhardt et al. (1994). In the diseased mammary gland in our experiment, a significant increase of AcP was found in the epithelial cells. The increased level of AcP probably reflects the raised lysosomal activity during chronic mastitis. This is in agreement with the observation of Kotz et al. (1978), who described a significant increase of AcP in the epithelial cells during acute mastitis and recommended the use of this reaction for the identification of mastitis intensity in the mammary gland.

In comparison with clinically healthy mammary glands, where a low activity of succinate dehydrogenase in all cellular structures was detected (Longauer and Bilčík, 1980; Lenhardt et al., 1994) in our experiment the most noticeable increase of this enzyme was found in the epithelial cells. In other structures, a lower activity of SDH was detected. This is in agreement with the results of Michel (1979a), where the activity of SDH detected in myoepithelial cells of the alveoli, stroma, and capillaries in the mastitic changed mammary gland was very low.

In conclusion, the densitometric quantification of the enzymatic activity between healthy and chronic mastitis in the mammary gland showed marked changes in the enzymatic activity of the epithelial cells. The use of this histochemical method should be one of the tools for the precise histochemical examination of acute and chronic mastitis as well as the picture of the functional status of the mammary gland.

REFERENCES


Michel G. (1979a): Das histochemische Verhalten der Sulzinatdehydrogenase und Laktatdehydrogenase sowie der Ribo-


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