EPITHELIAL AND NON-EPITHELIAL CELL- AND TISSUE CULTURE FROM THE RUMINAL MUCOSA

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Introduction

It is known that a considerable amount of nutrient substances and metabolites is transported across the ruminal epithelium, with the active involvement of its cells. The metabolic activities of the ruminal mucosa have been well established, but few methodical approach has as yet been described which would permit the direct study of the ruminal mucous cells. An experimental model based on the characterized cell and tissue culture of ruminal mucosa was developed and described in this paper.

Materials and Methods

Papillae were taken from the caudal blind sac of the rumen of healthy merino ewes (weighing about 40 kg) after slaughter and were digested by fractional trypsinization described earlier (Gálfi et al., 1981; Inooka et al., 1986). The epithelial cells obtained were cultured (Gálfi et al., 1981) on different collagen (Type I, IV) with or without Ca ionophore pretreatment (A23187, Kaneko and Goshima, 1982) and the cells grown from explants of the trypsinised papillae were cultured on plastic. The epithelial origin of the cells was proved by immunological detection of the ruminal carbonic anhydrase isoenzyme (RCA, Gálfi et al., 1981), cytokeratin (anti-cytokeratin-pan, LU5, reactive with keratins 1-19, Boehringer Mannheim GmbH, Germany) and examination of bacterial adherence *Streptococcus bovis* A1 (a generous gift from Dr. G. Semjén, Department of Pharmacology, University of Veterinary Sciences, Hungary). The morphology and the differentiation of the cells was investigated by light and electron microscopy (Gálfi et al., 1981; Adams and Watt, 1988) and identification of cornified envelope (Adams and Watt 1988). The cell division was determined by measuring of DNA synthesis (^{3}H-thymidine incorporation, Neogrády et al., 1989) and cell number (light microscopy) in the presence or absence of several chemicals added to the medium.

Results and Discussion

In the epithelial cell cultures two types of ruminal epithelial cells (REC) were distinguished: not keratinized cells (REC-NK) and keratinized cells (REC-K). In both cell types were detected RCA, LU5 and observed bacterial adherence. However they differed in some respects: the REC-NK grew in monolayer, its division was regulated by contact inhibition, produced microvilli in the course of differentiation, was not A23187 sensitive and didn't form cornified envelope; the REC-K grew in multilayer but only on REC-NK, produced microplicae, its division was reduced by A23187 and formed cornified envelope in the course of differentiation. Both cell types (strains) including the unusual not keratinized type were distinguished also from human epidermal keratinocytes (Adams and Watt, 1988).

In the cultures of ruminal connective tissue (RCT) also two cell types were detected and characterized by morphology: endodermal- and fibroblast-like cells. The cells of RCT didn't show positive reaction to RCA, LU5 and bacterial adherence didn't occur.

The division of REC NK and REC-K could be affected by different factors (table 1.). The rate of division was increased by treatment with some hormones (insulin, glucagon, pentagastrin, cortisol, prostaglandin $F_{2\alpha}$/$PGF_{2\alpha}$) and decreased by butyrate addition. In the presence of butyrate the formation of protrusion was observed on the REC-NK grown not confluent. The pretreatment by A23187 inhibited the proliferation of REC-K but not REC-NK. The process of keratinization (formation of cornified envelope in REC-K) was stimulated by cortisol, $PGF_{2\alpha}$, A23187 and butyrate. The extracellular matrix (collagen I, IV) and

TABLE 1. THE INFLUENCE OF CHEMICALS ON THE CELL DIVISION OF THE DIFFERENT RUMINAL MUCOUS CELLS

Treatment (M)	REC-NK		REC-K		REC-NK+REC-K	RCT
	Cell No I[a]	Cell No IV[a]	Cell No I[a]	Cell No IV[a]	^{3}HTdR in DNA[b]	Cell No
Insulin ($1.6x10^{-9}$)	157±67	ND	241±9** (80±30)[c]	85±8 (114±43)	328±12***	145±109
Glucagon ($3x10^{-10}$)	221±86	ND	187±86 (30±10)	106±16 (128±43)	126±13	446±72**
Pentagastrin ($2.5x10^{-5}$)	ND	ND	ND	ND	283±76**	ND
Cortisol (10^{-7})	121±79	ND	206±45 (110±30)	80±4 (300±171)	179±35**	172±118
$PGF_2\alpha$ (10^{-6})	129±36	ND	201±34 (120±50)	78±8 (314±71)*	ND	ND
A23187 ($3.8x10^{-5}$)	76±36	62±27	0 (40±20)	0 (57±57)	ND	ND
Butyrate ($5x10^{-3}$)	11±4**	6±1***	0 (60±40)	0 (114±43)	7±2**	18±11**

The cells (REC-NK, REC-K, RCT) in the presence of chemicals were cultured for 2 weeks. [a]The plastic dishes were covered or not by collagen (type I, IV). [b]The DNA synthesis was measured by ^{3}H thymidine (^{3}HTdR) incorporation on one week cultures (REC-NK, REC-K can't be distinguished), drawn after the data in Neogrády et al.(1989). Cell number in culture was measured by morphological method. [c]Detached cells having cornified envelope. Control was taken as 100%. Values are means±SD of three, four replicates as % of control. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (vs control value).

the presence of RCT cells also influenced the proliferation and differentiation.

The cells of RCT were examined in tissue cultures. It seems that these cells also differentiate in culture, but their sensitivity against hormones (e.g. rate of growth stimulation by glucagon) differs significantly from that of REC (table 1.).

It can be concluded that REC cultures could be appropriate tools for studying the physiological and biochemical processes taking place in the ruminal mucosa (epithelial transport: REC-NK and protective function in connection with keratinization: REC-K). In the tissue cultures of rumen mucosa the growth promoting interactions of different cell types (REC and RCT) can be investigated.

(Key Words: Ruminal Epithelial and Non-Epithelial Cells, Differentiation, Division)

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