Acta Veterinaria Hungarica 50 (2), pp. 211–215 (2002)

NA^{+}/H^{+} EXCHANGE IN PRIMARY, SECONDARY AND n-BUTYRATE-TREATED CULTURES OF RUMINAL EPITHELIAL CELLS: SHORT COMMUNICATION

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(Received January 10, 2002; accepted March 13, 2002)

Rate of amiloride-sensitive Na^{+} uptake into cultured rumen epithelial cells was studied in order to clarify the influence of culture conditions on Na^{+}/H^{+} exchange (NHE). Cell cultures were exposed to Na-n-butyrate or not for seven days or subcultured. On the 14th day of culturing, primary cell cultures without butyrate exposure showed both non-stratified and stratified growth. Na-n-butyrate treated 14-day-old cultures and 3-day-old subcultures contained mostly non-stratified, i.e. non-keratinised cells. Both n-butyrate treatment and subculturing increased total and amiloride-sensitive Na^{+} uptake. Our results indicate that Na^{+} uptake via NHE is determined by the amount and the ratio of non-stratified (non-keratinised) cells.

Key words: Ruminal epithelium, Na^{+} uptake, proliferation, differentiation, lectin

In an earlier study, we demonstrated that Na^{+}/H^{+} exchange (NHE) is detectable in rumen epithelial cells kept in primary culture (Gäbel et al., 1996). In the intact rumen epithelium, NHE plays a role in transcellular sodium transport (Martens et al., 1991) but it is also important in the intracellular pH regulation and cell proliferation or differentiation (Grinstein et al., 1989; Delvaux et al., 1990; Müller et al., 2000). However, in the primary cell cultures of ovine ruminal epithelium used in the earlier studies, NHE – the amiloride-sensitive type of Na^{+} uptake – amounted to only 30–40% of total Na^{+} uptake (Gäbel et al., 1996), while the rate of amiloride-sensitive Na^{+} transport was 85–90% in isolated ruminal epithelia of sheep (Martens et al., 1991). Despite distinct experimental conditions, dissimilar expression of transport proteins might be an underlying reason for the various rates of amiloride-sensitive Na^{+} uptake. Although the presence of Na^{+} channels can be most probably excluded (Gäbel et al., 1996), Na^{+}-coupled HCO_3^{-} transport may be involved in Na^{+} uptake. In our earlier studies, therefore,

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we tested the sensitivity of Na^+ uptake to 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS), an inhibitor of Na^+/HCO_3^--cotransport (Heyer et al., 1999; Sciortino and Romero, 1999). We found that DIDS did not inhibit Na^+ uptake into primary cultures of ovine ruminal epithelium (data not shown).

In the present study, we tried to evaluate whether variations of total and amiloride-sensitive Na^+ uptake are due to differences in the cell population examined. For this purpose, primary cell cultures of ovine ruminal epithelium were subjected to various treatments in order to influence the ratio of non-stratified to stratified colonies. Previous studies have shown that stratified growth is strongly correlated with a higher rate of differentiation (Gálfi et al., 1993). Standard procedure of isolation and cultivation of ruminal epithelial cells was described by Gálfi et al. (1993). Manipulation of differentiation procedure was conducted according to Gálfi et al. (1981, 1993). One-week-old cultures grown on collagen I coated dishes were treated for further seven days with 10 mmol/l Na-n-butyrate (culture B), a substance known to lead to detachment of stratified cells. On this ways, those cells were left on the culture dish which grew in non-stratified colonies. Furthermore, cells were subcultured according to Štyriak et al. (1992) to obtain young cells grew in non-stratified colonies (culture C) or subjected to freezing (–20 °C for 24 h, culture D). To evaluate the amount of differentiated (keratinised) cells, culture dishes were studied by the *Ulex europaeus* I lectin (UEA I lectin) binding test according to the previously described method (Gálfi et al., 1993). Only stratified colonies bound UEA I lectin (Gálfi et al., 1993).

Primary culture maintained for 14 days without butyrate exposure (culture A) contained both non-stratified and stratified colonies (Fig. 1a). Stratified areas were positive for UEA I lectin (Fig. 1g). In contrast, 14-day-old primary culture treated with Na-n-butyrate for 7 days (culture B) contained mainly non-stratified colonies (Fig. 1b). The cells showed projections and were negatively stained by UEA I lectin. (Fig. 1h). The 3-day-old secondary subcultures (culture C) contained mainly non-stratified colonies (Fig. 1c) and were negatively stained by UEA I lectin (Fig. 1i). According to culture A, 14-day-old primary cell cultures subjected to freezing (culture D) contained both non-stratified and stratified colonies (not shown in Fig. 1). Epithelial origin of cultures was determined by indirect immunofluorescence detection of cytokeratin as described earlier (Gálfi et al., 1993). As shown in Figs 1d, e and f, all cell cultures were positively labelled for cytokeratin.

Na^+ uptake was quantified by the method described by Gäbel et al. (1996). Uptake experiments were performed on four cultures from rumen epithelium of sheep in three replications. As shown in Fig. 2, total Na^+ uptake of culture A (primary culture for 14 days, cells grew in stratified and non-stratified colonies) amounted to 11.0 ± 0.5 nmol/(mg protein × 3 min). Total Na^+ uptake of culture B (Na-n-butyrate treatment for 7 days, contained mainly non-stratified colonies) was more than twice as high. In cell culture C (secondary culture with young

cells, grew in non-stratified colonies), the highest total Na^+ uptake was observed (Fig. 2). In the cultures B and C, Na^+ uptake was sensitive to amiloride. Amiloride-sensitive Na^+ uptake (that means the difference between Na^+ uptake in the absence of amiloride and Na^+ uptake in the presence of 2 mmol/l amiloride) of cultures B and C reached 81.6 ± 3.9% and 89.4 ± 0.8%, respectively, similarly as it was found in isolated rumen epithelium by Martens et al. (1991). To demonstrate the specific effect of amiloride on Na^+ uptake, primary cell cultures were maintained for 14 days and permeabilised by freezing in culture D. Freezing led to a drastic inhibition of total and amiloride-sensitive part of Na^+ uptake (Fig. 2). Freezing probably impaired the transport processes of cells, and thus also their Na^+ uptake.

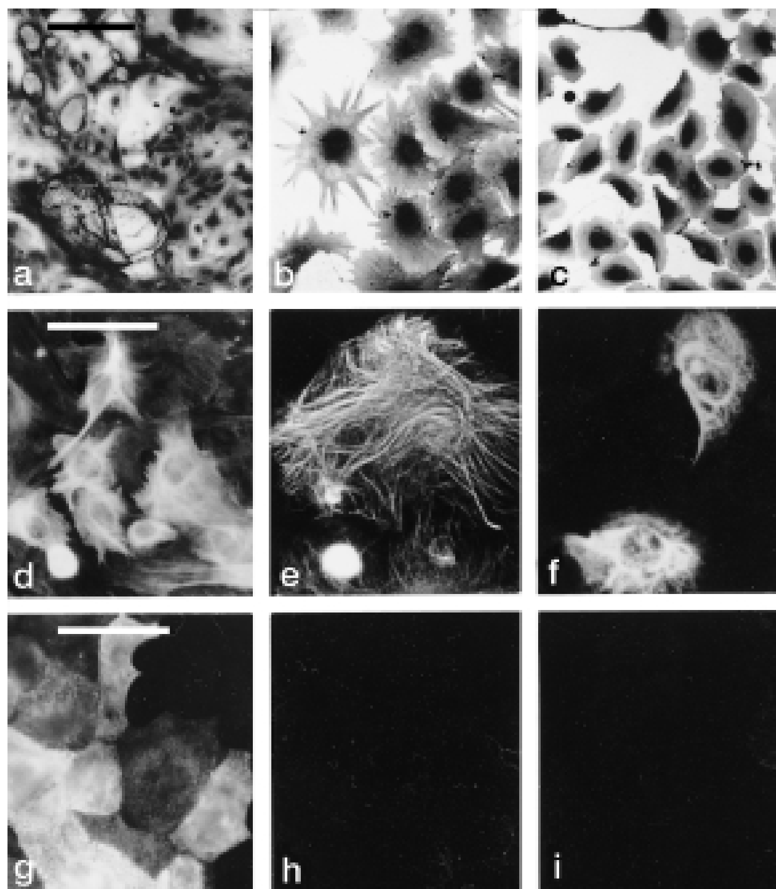

Fig. 1. Identification of ruminal epithelial cells in 14-day-old primary culture (a, d, g), in 14-day-old primary culture treated with 10 mmol/l Na-n-butyrate for one week (b, e, h) and in 3-day-old secondary culture (c, f, i). a–c: Giemsa staining, d–f: indirect immunohistochemical identification of keratins with anti-cytokeratin pan, LU5 monoclonal antibody, g–i: direct immunofluorescent staining of glycoproteins with UEA I-FITC lectin. Bars = 110 µm (a–c) or 70 µm (d–i)

These results solve the apparent contradiction mentioned before and indicate that NHE varies in accordance with the culture condition, hereby mainly with the ratio of non-stratified, non-keratinised cells, since it is localised mainly in the non-stratified areas as described earlier (Gäbel et al., 1996). However, it is not yet clear whether the changes in NHE activity merely accompany the differentiation process or if they are absolutely necessary for the triggering of differentiation or proliferation processes.

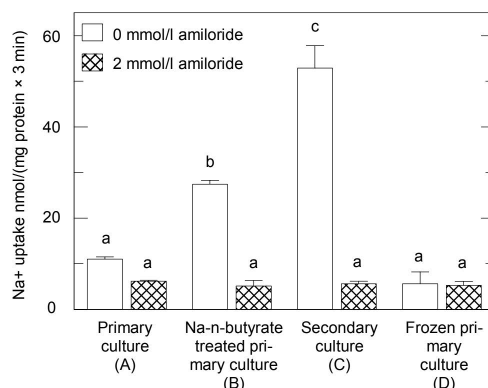

Fig. 2. Effect of pre-treatment on Na^+ uptake into ruminal epithelial cell culture in the presence or absence of 2 mmol/l amiloride. Values are expressed as means ± SEM of four separate experiments made in triplicate. Culture conditions are explained in the text. [a, b, c]Different letters denote a significant difference of at least $P < 0.05$ (Student-Newman-Keuls test)

Acknowledgement

This work was supported by the Hungarian Scientific Research Fund (OTKA), project no. T 026060.

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