7 Summary

A vH^+-ATPase
and its evidence for regulation of pH in sheep ruminal epithelial cells

The secretory and absorptive functions of the ruminal epithelium in ruminants depend on the existence of active transport mechanisms. In this context, it was recently confirmed that a vacuolar type H^+-ATPase (vH^+-ATPase) is expressed besides the Na^+/K^+-ATPase in ovine ruminal epithelial cells.
Therefore, the aim of this work was to determine the functional importance of the vH^+-ATPase for maintaining the intracellular pH (pH_i) as well as to identify some factors which regulate vH^+-ATPase activity.
We conducted spectrofluorometrical measurements on sheep ruminal epithelial cells (REC), which were incubated in nominally CO_2/HCO_3^--free media and loaded with the pH-sensitive dye BCECF. The contribution of vH^+-ATPase and Na^+/H^+-exchanger (NHE) subtype 1 and 3 activity to the regulation of pH_i was determined by using selective inhibitors (foliomycin, HOE694, S3226).

Our experiments delivered the following results:

1. The initial pH_i of REC was 7.43 ± 0.22 in HEPES-buffered NaCl medium and 7.02 ± 0.12 after acid loading with 20mM butyrate. The acidification induced by butyrate was followed by a pH_i recovery that amounted to 0.57 ± 0.05 pH-units per 10 min.
2. Using the selective H^+-ATPase inhibitor foliomycin (2µM) caused a reduction of pH_i in both media by roughly 0.2 pH-units and also lead to a reduced butyrate-induced pH_i recovery. The present results clearly demonstrate a functionally and quantitatively important vH^+-ATPase activity in ovine REC.

3. Specific inhibitors of the NHE subtype 1 (HOE694) and of the NHE subtype 3 (S3226) were applied in addition to foliomycin. This reinforced the effects on the pH_i (-0.64 ± 0.2 pH-units) as well as on the pH_i-recovery (-0.2 ± 0.1 pH-units/10 min).
4. On the basis of the inhibitor studies that we conducted, we found under nominally HCO_3^--free conditions that vH^+-ATPase activity contributes for about 30 %, NHE1 for about 50% and NHE3 for about 20% on H^+ extrusion in REC.
5. These results confirm the dominant role of NHE1 for the pH_i-recovery after an acid load under HCO_3^--free conditions.
6. Incubation of REC in a medium with a reduced extracellular Cl^--concentration (36 mM instead of 136 mM) led to a significant reduction of pH_i (-0.51 ± 0.03 pH-units) as well as of the butyrate-induced pH_i recovery (0.38 ± 0.05 instead of 0.57 ± 0.04 pH-units/10 min). Moreover, the inhibitory effect of Foliomycin was abolished and the parts of pH_i sensitive to HOE694 and S3226 were smaller in the solution with reduced $[Cl^-]$.
7. These findings show the fact that the extracellular $[Cl^-]$ influences the functional activity of important H^+-excreting mechanisms (vH^+-ATPase, NHE) via hitherto unknown ways. An influence of $[Cl^-]_e$ on the NHE activity in REC was first shown in this study.