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Intracellular enzymatic trapping and degradation prevent transport of intact [^{14}C] adenosine across the sheep choroid plexus epithelium as a monolayer in primary culture

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Efflux transport of adenosine across the choroid plexus (CP) epithelium might contribute to the homeostasis of this neuromodulator in the extracellular fluids of the brain. The aim of this study was to explore adenosine transport across sheep CP epithelial cell monolayers in primary culture. We used a method for primary culture of the sheep choroid plexus epithelial cells (CPEC) on plastic permeable supports and analysed [^{14}C] adenosine transport across this cellular layer, metabolism inside the cells and cellular uptake of [^{14}C] adenosine from either of the chambers. Primary cultures of CPEC were established using the choroid plexus from the IVth ventricle of sheep. CPEC expresses some features typical of the CPEC *in situ*, including three nucleoside transporters at the transcript level that normally mediate adenosine transport across cellular membranes. The estimated permeability of these monolayers towards [^{14}C] adenosine was low and the same order of magnitude as for the markers of paracellular diffusion. However, inhibition of the intracellular enzymes, adenosine kinase and adenosine deaminase, led to a significant increase ($p>0.01$ by ANOVA) in transcellular permeability, indicating that intracellular phosphorylation into nucleotides might be a reason for the low transcellular permeability. HPLC analysis with simultaneous detection of radioactivity revealed that [^{14}C] radioactivity which appeared in the acceptor chamber after the incubation of CPEC monolayers with [^{14}C] adenosine in the donor chamber was mostly present as [^{14}C] hypoxanthine, a product of adenosine metabolic degradation. Therefore, it appears that CPEC in primary cultures act as an enzymatic barrier towards adenosine. Cellular uptake studies revealed that concentrative uptake of [^{14}C] adenosine was confined only to the side of these cells facing the upper or apical chamber, indicating uneven distribution of nucleoside transporters.

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