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Production and Characterization of Multiple Antigenic Peptide Antibodies to the Adenosine A_{2b} Receptor

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SUMMARY

A polyclonal antibody to the human adenosine A_{2b} receptor ($A_{2b}R$) was produced by immunizing a chicken with a multiple antigenic peptide consisting of eight copies of a 16-amino acid peptide, corresponding to the presumed second extracellular loop of the $A_{2b}R$, linked to a branched lysine core. Western blotting with affinity-purified antibody revealed the human $A_{2b}R$ to be a protein of approximately 50–55 kDa, found in a variety of tissues including thymus, colon, and small intestine. The antibody also recognized mouse and rat $A_{2b}Rs$ and revealed heterogeneity in size, with a 35-kDa protein being detected in small intestine in addition to the larger 50–52-kDa species in thymus, colon, and placenta. The chicken anti-human $A_{2b}R$ peptide antibody recognized the receptor in both frozen and formalin-fixed tissue sections. In human colon, the $A_{2b}R$ was highly expressed in epithelial cells of the crypts. $A_{2b}R$ immunoreactivity was also apparent in syncytiotrophoblast cells of human placental villi and in the basal zone of murine chorioallantoic placenta. These cell type-specific patterns of expression are consistent with the hypothesized roles of the $A_{2b}R$ in mediating electrogenic Cl^- secretion and the resulting secretory diarrhea caused by colonic crypt abscesses and in regulating morphogenesis of the placenta. Insight into the multiple physiological consequences of $A_{2b}R$ engagement will be forthcoming from an analysis of the cell type-specific expression of this receptor in additional tissues.

ARs are members of the large family of G protein-coupled receptors whose activation on the cell surface can influence signal transduction events through cAMP and ion fluxes (1). These receptors have diverse input into many important physiological responses, including platelet aggregation, cardiac rate, smooth muscle tone, inflammation, neurotransmission, and cell growth and death (2–6). Considerable interest in the classification of AR subtypes in specific tissues derives largely from the therapeutic potential of selective and nonselective AR agonists and antagonists (7). Currently, four AR subtypes are known (A_1, A_{2a}, A_{2b}, and A_3), which differ in their affinity for adenosine and in their tissue distribution (8–11).

The $A_{2b}R$ has a low affinity for adenosine (12) and is poorly characterized pharmacologically and physiologically. Northern blot analysis has demonstrated pronounced expression of $A_{2b}R$ transcripts in tissues such as brain, spinal cord, caecum, large intestine, and urinary bladder (13). More recently, high-level expression of $A_{2b}R$ transcripts has been detected in the T84 human intestinal cell line (14) and in the murine gestation site (15). The T84 cell line displays colon crypt-like features. Stimulation by neutrophil-derived adenosine of the apical membrane $A_{2b}R$ of these cells leads to an electrogenic Cl^- efflux and a secretory response, providing a model for the secretory diarrhea seen in various inflammatory intestinal disorders (14, 16). Dynamic expression of the $A_{2b}R$ has also been demonstrated in the early postimplantation uterus of pregnant mice and in the ectoplacental cone of developing mouse embryos. Engagement of ARs in these tissues may serve as a transitory maternal implantation signal that delays ectoplacental outgrowth until the enzymatic differentiation of the secondary decidua has occurred (15).

Due to the lack of widely available agonists and antagonists specific for the $A_{2b}R$ and the limitations of *in situ* hybridization, we were motivated to generate an immunolog-

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ABBREVIATIONS: AR, adenosine receptor; BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; HRP, horseradish peroxidase; MAP, multiple antigenic peptide; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; ADA, adenosine deaminase; PCR, polymerase chain reaction; bp, base pair(s); PAGE, polyacrylamide gel electrophoresis.