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Characterization of a P2Y2 nucleotide receptor antibody by Western blot analysis

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P2 nucleotide receptors modulate a wide range of physiological responses following their activation by extracellular nucleotides (Ralevic V et al., Pharmacol. Rev. 1998; 50: 413-492). The G protein-coupled $P2Y_2$ nucleotide receptor ($P2Y_2R$) subtype is fully activated by equivalent concentrations of ATP or UTP and is up-regulated in salivary gland models of stress and disease (Turner JT et al., Am. J. Physiol. 1997; 273: C1100-C1107; Ahn JS et al., Am. J. Physiol. 2000; 279: C286-C294; Schrader AM et al., Arch. Oral. Biol. 2005; 50: 533-540), in blood vessels after balloon angioplasty, and in collared carotid arteries where they promote intimal hyperplasia and inflammation by increasing smooth muscle cell proliferation and leukocyte infiltration (Seye CI et al., Arterioscler. Thromb. Vasc. Biol. 1997; 17: 3602-3610; Seye CI et al., 2002; Circulation 106: 2720-2726). Since a reliable anti-$P2Y_2R$ antibody is not currently available, determination of the presence of the $P2Y_2R$ in cells and tissues has been limited to $P2Y_2R$ mRNA quantification by reverse transcription-polymerase chain reaction (RT-PCR) or in situ hybridization of cells or tissues using $P2Y_2R$-specific riboprobes. Alternatively, the functional activity of the $P2Y_2R$ in freshly isolated cells or established cell cultures can be determined by measuring changes in the intracellular free calcium concentration in response to ATP or UTP. Recently, a commercially-available anti-rat $P2Y_2R$ antibody has been produced by Alamone Laboratories (Jerusalem, Israel). The purpose of this study is to characterize the specificity of the Alamone antibody for the $P2Y_2R$ in human, rat and mouse tissues. Preliminary results from Western blot analysis of cell lysates from the rat ParC10 salivary gland cell line that expresses endogenous $P2Y_2Rs$ indicate a single band with an approximate size of 45 kD. Furthermore, a primary preparation of rat submandibular gland acinar cells cultured for 48 h also yielded a 45 kD band in Western analysis, whereas freshly prepared (0 time) acini did not show any bands, consistent with the observation that the $P2Y_2R$ is upregulated in submandibular gland acini as a function of time of culture. Additional experiments are underway to evaluate the specificity of the antibody with cells from $P2Y_2R$ knock-out mice and human 1321N1 astrocytoma cells expressing the recombinant human $P2Y_2R$.