Abstract Four adenosine receptors have been cloned from many mammalian and some non-mammalian species. In each case the translated part of the receptor is encoded by two separate exons. Two separate promoters regulate the A1 receptor expression, and a similar situation may pertain also for the other receptors. The receptors are expressed in a cell and tissue specific manner, even though A1 and A2B receptors are found in many different cell types. Emerging data indicate that the receptor protein is targeted to specific parts of the cell. A1 and A3 receptors activate the G i family of G proteins, whereas A2A and A2B receptors activate the G s family. However, other G proteins can also be activated even though the physiological significance of this is unknown. Following the activation of G proteins several cellular effector pathways can be affected. Signaling via adenosine receptors is also known to interact in functionally important ways with signaling initiated via other receptors.

Key words Adenosine receptors · Transcriptional regulation · G proteins · Signal transduction · Phosphorylation

Introduction

This short overview focuses on aspects not covered by several recent reviews and monographs (Linden 1991; Cronstein 1994; Williams 1994; Olah and Stiles 1995; Fredholm 1996, 1997; Sebastiao and Ribeiro 1996; Fredholm et al. 1998; Svenningsson et al. 1999b). In this review we only use the term adenosine receptor and not the term P1 receptor because the concept of adenosine receptors precedes the later concept of purinoceptors (P1 and P2; Burnstock 1978) by several years. After the discovery by Drury and Szent-Györgyi (1929) that adenosine can influence several bodily functions the pronounced cardiovascular effects of adenosine were particularly well investigated. Several adenosine analogues were synthesized and examination of the dose-response relationships suggested the presence of specific adenosine receptors (Cobb et al. 1974). The essentially competitive nature of the antagonism by methylxanthines of adenosine effects in the heart (De Gubareff and Sleator 1965) and also in the brain (Sattin and Rall 1970) also supported the idea of adenosine receptors. We also do not use the concept of purinoceptors as it has now been superseded (Fredholm et al. 1997). It can also be mentioned that the P1 receptor was defined as a receptor inhibited by methylxanthines, activated weakly by some adenine nucleotides, affecting changes in cAMP and not affecting synthesis of prostaglandins (Burnstock 1980). Later developments have shown that none of these criteria are very useful. Instead the primary basis for defining adenosine receptors is structural.

Purification and cloning of adenosine receptors

Once the adenosine A1 receptor was conclusively defined using binding assays, several attempts were made to purify the receptor. Although much progress was made, the receptor was never obtained in a sufficiently high purity to allow sequencing. Instead, the cloning of the first adenosine receptors was serendipitous. Four novel members of the G protein coupled receptor family were cloned from a canine thyroid library (Libert et al. 1989). Of these one turned out to be the adenosine A2A receptor (Maenhaut et al. 1990), another the canine A1 receptor (Libert et al. 1991). Once these first structures were obtained, the same receptors were soon cloned from other mammals including man. Furthermore, the adenosine A2B receptor
was cloned (Stehle et al. 1992). More surprisingly a fourth adenosine receptor, denoted A3, was cloned, first as an orphan (Meyerhof et al. 1991), later as a bona fide, methylxanthine-insensitive, adenosine receptor (Zhou et al. 1992).

Differences in structure

By now all four adenosine receptors have been cloned from rat, mouse and man. In addition, A1 receptors are cloned from dog, cow, rabbit, guinea pig and chick; the A2A receptor from dog and guinea-pig, the A2B receptor from chick, and the A3 receptor from dog, sheep and chick. As seen from the dendrogram in Fig. 1, there is a close similarity in structure between the receptors within a sub-type, at least for mammals. The largest variability is observed for the A3 receptor, for which there is almost a 30% difference at the amino acid level between human and rabbit. This difference is in fact larger than that seen between human and chick for the A1 receptor. As seen from the article by Klotz (2000), there is also a very large difference between A3 receptors from different species with regard to ligand specificity.

Much work has been done to elucidate the amino acids responsible for binding of receptor agonists. It seems clear that TM3 and TM7 are very important (Kim et al. 1995; Rivkees et al. 1999a). This also agrees with data from other G protein coupled receptors (Gether and Kobilka 1998; Wess 1998). In Fig. 2 we highlight the conserved amino acids in TM3 and TM7, particularly those implicated to be important in agonist binding by site-directed mutagenesis studies.

Genomic structure

The exon-intron structure appears to be similar for all the human adenosine receptors (see Fig. 3). The best-studied receptor is the A1 receptor. There is a single intron that interrupts the coding sequence in a region corresponding to the second intracellular loop (Ren and Stiles 1994). Already when the structure of the A1 receptor was first reported the presence of two major transcripts was noted. It was originally thought that they may represent alternative splicing, but more recent data have revealed another more unusual explanation (Ren and Stiles 1994, 1995). There are two promoters, a proximal one denoted promoter A, and a distal one denoted promoter B, which are about 600 bp apart (see Fig. 4). Promoter B and exon 1B are part of an intron when promoter A is active (Ren and Stiles 1995). Both promoters were suggested to have non-traditional TATA-boxes.

Reporter assay studies in DDT1 MF-2 cells show that 500 base pairs of promoter A contained essential elements for A1 receptor expression, and mice expressing promoter