

## A Pharmacological Characterization of Adenosine Receptors

Nouh MH. Aljarari<sup>1</sup>, Dhastagir Sultan Sheriff<sup>2\*</sup>

1. Department of Pharmacology, Faculty of Medicine, Benghazi University, Benghazi-Libya

2. Department of Biochemistry, Faculty of Medicine, Benghazi University, Benghazi-Libya

**\*Corresponding author:** Dr.Dhastagir Sultan Sheriff, Department of Biochemistry, Faculty of Medicine, Benghazi University, Benghazi, Libya. E.mail: drdsheeriff@gmail.com

Received 26 September; accepted 19 October; published online 01 November; printed 15 November 2012

## ABSTRACT

Adenosine receptors (AR) are member of the G-protein Coupled Receptors (GPCR) superfamily, with four subtypes currently recognised, A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> and A<sub>3</sub> receptors. Because of various potential physiological implications of stimulating AR, the main purpose of the present review was to briefly describe the pharmacological properties of these receptors and how they could be activated. Since these receptors are G-protein-coupled receptors, and so exert their effects by coupling to heterotrimeric G proteins, it is important to follow the G proteins functions and how they regulate the intracellular response of activated receptors, as well as their ability to couple to multiple second messenger signaling pathways. The responses to adenosine (ADO) are governed by the selective activation of distinct G proteins by AR subtypes. The A<sub>2A</sub> and A<sub>2B</sub> both couple via G<sub>s</sub> to adenylyl cyclase (AC) stimulation (A<sub>2B</sub> can also couple to G<sub>i</sub> subtype), while the A<sub>1</sub> and A<sub>3</sub> are mainly coupled to G<sub>i</sub> protein subtype to G<sub>i</sub> to inhibit AC (although coupling via G<sub>o</sub> and G<sub>q/11</sub> respectively). A<sub>1</sub> and A<sub>2A</sub> are considered high affinity receptors while A<sub>2B</sub> and A<sub>3</sub> receptors are considered as low affinity receptors. Because of its potent actions on many organs and systems, adenosine is an obvious target for the development of new drugs.

**Key words:** Adenosine (ADO), Adenosine Receptors (AR), G protein coupled receptors (GPCR), AR subtypes.**Abbreviations:** ADO – Adenosine; AR - Adenosine Receptors; GPCR - G protein coupled receptors; cAMP - Cyclic adenosine 3,5 monophosphate; CPA - N6-cyclopentyladenosine; MAPK - Mitogen activated protein kinase; PSVT - Paroxysmal supraventricular tachycardia; HEL - human erythroleukaemia.

## 1. BACKGROUND

The diversity in intracellular signalling downstream of adenosine receptors (AR) is dependent on the receptor subtype activated by adenosine (ADO). Under physiological conditions ADO is shown to be present extracellularly at concentrations that can stimulate both the higher affinity A<sub>1</sub> and A<sub>2A</sub>. Under pathological conditions, ADO rises to concentrations that can also stimulate the lower affinity A<sub>2B</sub> and A<sub>3</sub>. Because AR are G-protein Coupled Receptors (GPCR), the conventionally-accepted mechanism is that, in their resting stage, G proteins exist as heterotrimers, with GDP bound to  $\alpha$  subunit (Figure 1). Following the occupying of the receptor by an agonist molecule, a conformational change occurs, involving the cytoplasmic domain of the receptor, which causes the G protein to bind to it (Neves et al., 2002), as the  $\alpha\beta\gamma$  trimer. Association of the  $\alpha\beta\gamma$  trimer with receptor causes the bound GDP to be replaced with GTP which, in turn, causes dissociation of the G-protein trimer, releasing  $\alpha$ -GTP and  $\beta\gamma$  subunits; these are the

active form of the G-protein (Hamm, 1998), which diffuse in the membrane. Dissociation of these subunits mediates diverse physiological responses via their effect on certain types of intracellular target and influencing the cellular signalling events through the second messenger pathways (Figure 1). The  $\alpha$ -subunit has intrinsic GTPase activity, which provides a self-limiting mechanism to G protein-mediated responses; once the GTP bound to the  $\alpha$  subunit has been hydrolysed to GDP, the  $\alpha$  subunit re-associates with  $\beta\gamma$  subunits, thus completing the G protein cycle (Rang et al.2003).

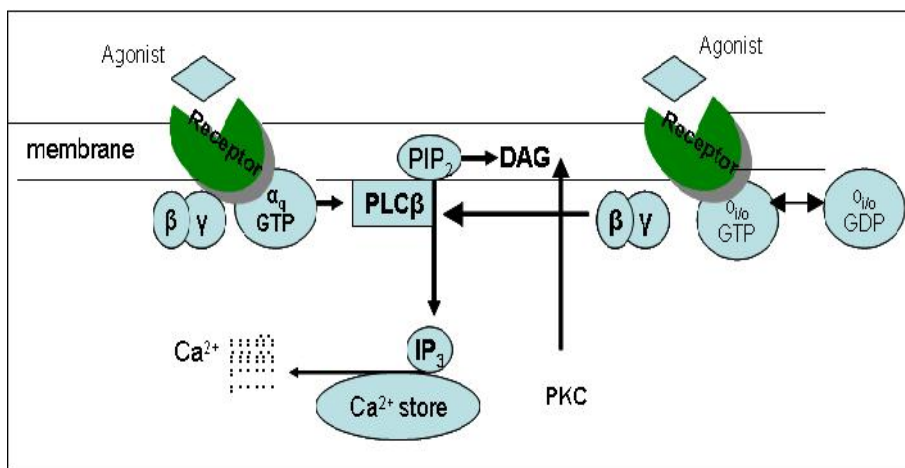
## 2. SIGNAL TRANSDUCTION OF ADENOSINE RECEPTORS ACTIVATION

The A<sub>1</sub> and A<sub>3</sub> are mainly coupled to G<sub>i</sub> protein subtype (although coupling via G<sub>o</sub> and G<sub>q</sub> respectively, have also been described) (Fredholm et al., 1994), while A<sub>2A</sub> and A<sub>2B</sub> can couple to G<sub>s</sub> or to G<sub>o/r</sub> subtype. As stated below

**Receptor:** A molecular structure or site on the surface or interior of a cell that binds with substances such as hormones, antigens, drugs, or neurotransmitters.

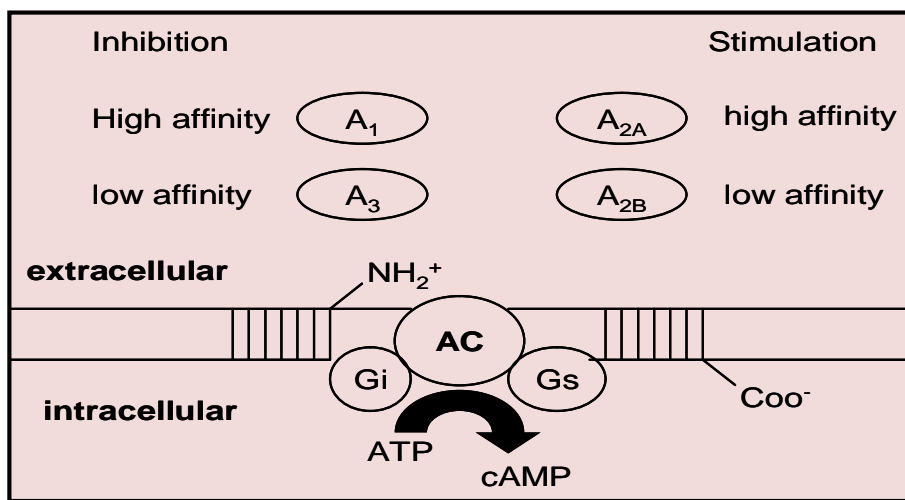
## G protein coupled receptors (GPCR)

G-protein-coupled receptors (GPCRs) constitute a large and diverse family of proteins whose primary function is to transduce extracellular stimuli into intracellular signals. They are among the largest and most diverse protein families in mammalian genomes. On the basis of homology with rhodopsin, they are predicted to contain seven membrane-spanning helices, an extracellular N-terminus and an intracellular C-terminus. This gives rise to their other names, the 7-TM receptors or the heptahelical receptors. GPCRs transduce extracellular stimuli to give intracellular signals through interaction of their intracellular domains with heterotrimeric G proteins, and the crystal structure of one member of this group, bovine rhodopsin, has recently been solved. The presence of GPCRs in the genomes of bacteria, yeast, plants, nematodes and other invertebrate groups argues in favor of a relatively early evolutionary origin of this group of molecules. The diversity of GPCRs is dictated both by the multiplicity of stimuli to which they respond, as well as by the variety of intracellular signalling pathways they activate. These include light, neurotransmitters, odorants, biogenic amines, lipids, proteins, amino acids, hormones, nucleotides, chemokines and, undoubtedly, many others. In addition, there are at least 18 different human G $\alpha$  proteins to which GPCRs can be coupled. These G $\alpha$  proteins form heterotrimeric complexes with G $\beta$  subunits, of which there are at least 5 types, and G $\gamma$  subunits, of which there are at least 11 types.



**Figure 1**

The conventional structure of GPCR with their coupling effectors. The adenosine receptors have seven transmembrane domains ( $\alpha$  helices), and have the ability to couple to G proteins. The COOH is believed to be intracellular while NH<sub>2</sub> remains extracellular. The G protein consists of a trimer ( $\alpha$ ,  $\beta$  &  $\gamma$ ), each of which has specific roles to activate its target effector, with  $\alpha$  subunit being able to bind and switch between GTP and GDP. When the agonist binds to the receptor, the G protein replaces GDP with GTP and  $\alpha$  subunit dissociates from  $\beta\gamma$  dimer, and bind to effector (AC). Also activation of PLC $\beta$  by adenosine receptors. Both G<sub>q</sub> and G<sub>i/o</sub> proteins regulate the function of PLC $\beta$ . The G<sub>s</sub> subunit of G<sub>q</sub> activates PLC directly, whereas G $\beta\gamma$  subunits typically released from G<sub>i/o</sub> also activate PLC. Hydrolysis of PIP<sub>2</sub> generates DAG and IP<sub>3</sub>.



**Figure 2**

A summary of the adenosine receptor classification with indicated coupling and affinities. Among the four AR subtypes, the A<sub>1</sub>- & A<sub>3</sub> are negatively coupled to AC via G<sub>i</sub> and/ or G<sub>o</sub> while A<sub>2A</sub>- & A<sub>2B</sub> are positively coupled to AC via G<sub>s</sub>. Consequently, the decrease or increase in cAMP levels leads to either inactivation or activation of protein kinase A (PKA), respectively (Hancock, 2005-9)

(Figure.2), A<sub>1</sub> and A<sub>2A</sub> are considered high affinity receptors while A<sub>2B</sub> and A<sub>3</sub> receptors are considered as low affinity receptors. It seems that A<sub>2B</sub> and A<sub>3</sub> are much more active in pathophysiological events when extracellular ADO concentration rises above the normal concentrations. The most widely recognized primary signalling mechanism of A<sub>1</sub> receptors is inhibition of adenylyl cyclase (AC) causing a decrease in cyclic adenosine 3,5 monophosphate (cAMP) (Cooper et al., 1980). The other signalling pathways of A<sub>1</sub> receptors are activation of PLC resulting in an increase in IP<sub>3</sub>, DAG and calcium mobilization (Megson et al., 1995). In addition, the A<sub>1</sub> receptors is coupled to activation of ATP-sensitive K<sup>+</sup> channels (K<sub>ATP</sub> channel) in guinea pig ventricular myocytes (Ito et al., 1994), and inhibition of calcium channels (Ca<sup>2+</sup>) has been described in dorsal root ganglion neurones (Sweeney & Dolphin, 1995), both of which inhibit neuronal activity. The protective effect of ADO during ischemia has been reported to be mediated primarily via the A<sub>1</sub> receptors (Matherne et al., 1997).

Conventionally A<sub>3</sub> receptors are coupled to G<sub>i</sub> or G<sub>o</sub> and also interact with G<sub>q/11</sub> subtypes of G protein (Palmer et al., 1995), and the classic second messenger system is inhibition of AC with a consequent decrease in cAMP accumulation (Figure 3). Other signalling pathways are also involved, including an increase in PLC (Abbracchio et al., 1995) and PLD activity (Ali et al., 1996), and the elevation of IP<sub>3</sub> levels, [Ca<sup>2+</sup>]<sub>i</sub> and the activation of PKC. The A<sub>3</sub> receptors has also been shown to regulate chloride channels (Mitchell et al., 1999). Although the accepted signal cascade of both A<sub>2A</sub> receptors (Olah, 1997) and A<sub>2B</sub> receptors (Olah & Stiles, 1995) is to increase cAMP generation via a positive coupling to AC by G<sub>s</sub> protein, there is increasing evidence to demonstrate that it is not the only second messenger pathway that can be activated by these subtypes (Fredholm et al., 2000). In human endothelial cells, Mitogen activated protein kinase (MAPK) (eg ERK, (SAPK) have been shown to be activated by A<sub>2A</sub> receptors through a tyrosine kinase, which appears to play an important role in cell differentiation, proliferation and death (Sexl et al., 1997) The activation of PLC with stimulation of IP<sub>3</sub> levels and calcium elevation has been observed to be a further signal transduction pathway by which the A<sub>2B</sub> receptors may evoke the cellular response. For instance, NECA was found to stimulate an increase in [Ca<sup>2+</sup>]<sub>i</sub> and cAMP accumulation in the human mast cell HMC-1 cells (Linden et al., 1999). In human erythroleukaemia (HEL) cells, activation of A<sub>2B</sub> receptors evoked a Ca<sup>2+</sup> influx, apparently not related to PIP<sub>2</sub> hydrolysis (IP<sub>3</sub>), but via a pertussis toxin-insensitive and cholera toxin-sensitive G protein coupling (G<sub>s</sub>) (Feoktistov et al., 1994). In contrast, in human T lymphocytes, A<sub>2B</sub> receptors activation by NECA results in cAMP generation, with no alteration in [Ca<sup>2+</sup>]<sub>i</sub> (Mirabet et al., 1999). Similarly, in HEK293 cells transfected with A<sub>2A</sub> receptors, CGS21680 (A<sub>2A</sub> selective agonist) produced an increase in cAMP accumulation without changing [Ca<sup>2+</sup>]<sub>i</sub> levels (Furlong et al., 1992).

**2.1. MAPK Pathways Adenosine receptors**

**2.1.1. p42/p44 (ERK1/2) Cascade**

Activation of the ERK1/2 (p42/44 MAPK) cascade pathway via adenosine receptors has been intensively studied in a variety of systems (Figure 4). For example, the selective A<sub>1</sub> agonist N6-cyclopentyladenosine (CPA) has been reported to increase MAP kinase activity in CHO-A1 cells (Dickenson et al., 1998). CGS21680 has also been reported to stimulate MAP kinase activity in HEK293 and CHO cells transfected with A<sub>2A</sub>AR (Seidel et al., 1999). The stimulation of endogenous A<sub>2B</sub>ARs by NECA has also been suggested to evoke activation of ERK1/2 in HEK293 cells (Gao et al., 1999), and in HMC-1 (Feoktistov et al., 1999). CHO3 cells have also been reported to produce concentration-dependent increases in ERK1/2 MAPK in response to IB-MECA, an A<sub>3</sub> high affinity agonist (Graham et al., 2001). The diverse effects of ADO on mitogenesis, depending on the receptor subtype activated, may be related to changes in MAPK. For instance, all the human AR transfected into CHO cells are able to activate ERK1/2 at physiologically relevant concentrations of the endogenous agonist ADO as well as its analogue NECA (Schulte & Fredholm, 2000). Interestingly in this study, NECA acting on A<sub>2B</sub> was much more potent in stimulating ERK1/2 phosphorylation (EC<sub>50</sub> = 19 nM) than cAMP formation (EC<sub>50</sub> = 1.4  $\mu$ M). While this report supports the accumulated evidence of activation of MAPK activation via all adenosine receptors, it was

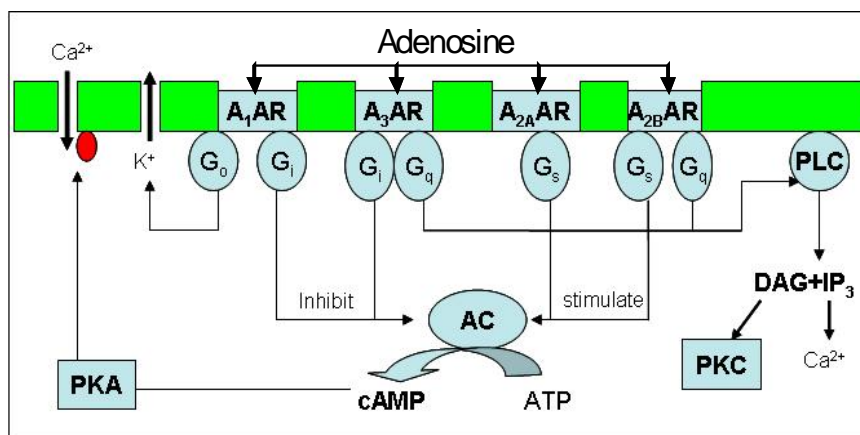


Figure 3

Signal transduction pathways associated with the activation of the human adenosine receptors. All AR are coupled to AC, A<sub>2A</sub> & A<sub>2B</sub> via G<sub>s</sub> while A<sub>1</sub> & A<sub>3</sub> via G<sub>i</sub>. Activation or inhibition of this pathway results in either increase or decrease of cAMP and subsequent stimulation or inhibition of PKA, respectively. A<sub>2B</sub> & A<sub>3</sub> are also coupled to PLC via G<sub>q</sub>. Activation of this pathway results in increase in DAG and IP<sub>3</sub>. DAG stimulates PKC. IP<sub>3</sub> activates mobilization of calcium from intracellular stores. A<sub>2B</sub> potentiate calcium influx directly by coupling with G<sub>s</sub>, and via cAMP and activation of PKA. A<sub>1</sub> are also coupled via G<sub>o</sub> to K<sup>+</sup> channel.

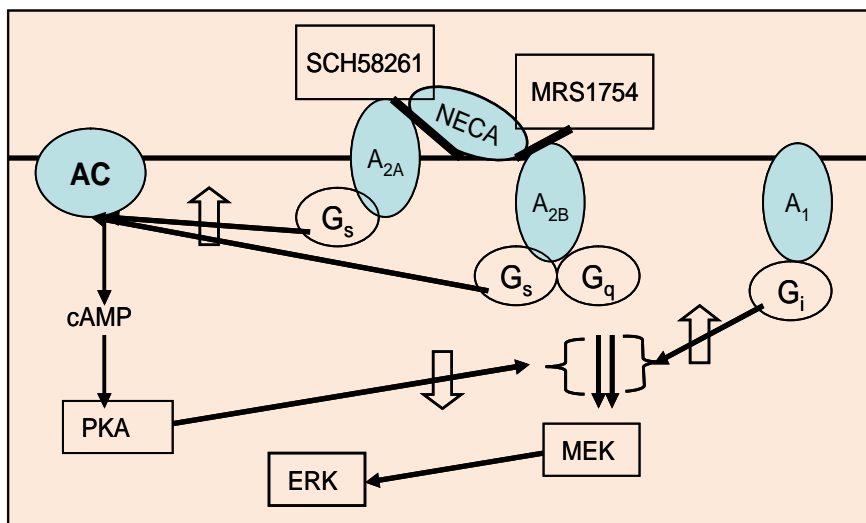


Figure 4

The ERK phosphorylation pathways via AR. It appears that involvement of the G<sub>s</sub>-coupled A<sub>2A</sub> in reduction of ERK phosphorylation via increase of AC and PKA activity (can be blocked by either A<sub>2A</sub> antagonist [SCH58261] or PKA inhibitor [H89]), while A<sub>2B</sub> receptors enhance ERK activity (can be inhibited by A<sub>2B</sub> antagonist [MRS1754] and MEK inhibitor [PD98059]).

conducted in transfected CHO cells and not cells with natively expressed receptors.

2.1.2 p38& JNK (SAPK) Cascade

These pathways are activated by interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α) and stress (heat) and is suggested to be involved in regulation of gene expression and cell differentiation (Seger & Krebs, 1995). Stimulation of A<sub>2B</sub>AR via NECA has been reported to regulate the ERK, JNK and p38 MAPK signalling cascades in HMC-1 cells (Feoktistov et al., 1999) and p38 MAPK phosphorylation in porcine coronary smooth muscle (Teng et al., 2005). A<sub>1</sub> stimulation has also been shown to activate both ERK1/2 and p38 phosphorylation in DDT1MF-2 cells (Robinson & Dickenson, 2001), and p38 in pig myocardial stunning (Yoshimura et al., 2004).

3. ADENOSINE RECEPTOR AGONISTS

Because of various potential physiological implications of stimulating ARs, a large number of agonists have been

synthesized (Table 1); the vast majority of them are structurally related to the physiological agonist ADO. The classic non-selective adenosine receptor agonist NECA stimulates A<sub>2B</sub>AR with low potency, 2 μM, but shows greater potency for other subtypes, high nanomolar (A<sub>3</sub>) or low nanomolar (A<sub>1</sub> & A<sub>2A</sub>) (Feoktistov & Biaggioni, 1997). Many A<sub>1</sub> agonists have been synthesized, with N<sup>6</sup>-substituted ADO derivatives being the most potent and selective, with K<sub>i</sub> values in the range of 0.6 to 1.3 nM, which include N<sup>6</sup>-cyclopentyladenosine (CPA), N<sup>6</sup>-cyclohexyladenosine (CHA), and R-PIA (Jacobson et al., 1992). Both IB-MECA and its derivative 2CI-IB-MECA are considered as selective agonists for human A<sub>3</sub>AR. 2CI-IB-MECA is considered as a highly selective agonist (K<sub>i</sub> = 0.33 nM) for A<sub>3</sub>AR, with 2500 and 1400 fold selectivity versus A<sub>1</sub> and A<sub>2A</sub>AR, respectively (Kim et al., 1994).

The distinction between high-affinity A<sub>2A</sub> and low-affinity A<sub>2B</sub> mediated responses was confirmed by the introduction of the ADO analogue CGS21680, which binds with nanomolar affinity to A<sub>2A</sub> (Jarvis et al., 1989) and does not bind to A<sub>2B</sub> up to micromolar concentrations (Stehle et al., 1992). CGS21680 is also 180 fold selective towards A<sub>1</sub> (Jarvis et al., 1989) and is unable to displace binding to A<sub>3</sub> receptors at micromolar concentrations (Zhou et al., 1992). CGS21680 is therefore, considered to be a prototypical A<sub>2A</sub> agonist, and is used pharmacologically to define the A<sub>2A</sub> subtype. However, there is a problem using CGS21680 as an A<sub>2A</sub> agonist & pharmacological tool, because it has been previously reported that it also binds to sites unrelated to A<sub>2A</sub> (Johansson et al., 1993; Lindstrom et al., 1996). Highly selective and potent agonists have been designed for A<sub>1</sub>, A<sub>2A</sub> & A<sub>3</sub>, however, no selective agonist has been found so far, and only non-selective agonists are available for A<sub>2B</sub>, and NECA is currently the most potent agonist at this subtype, having low micromolar affinity (Alexander et al., 1996; Klotz et al., 1998). Because it is non-selective, therefore, it is less useful in the characterization of A<sub>2B</sub> in cells or tissues in which A<sub>2A</sub> are also expressed.

4. ADENOSINE RECEPTOR ANTAGONISTS

Potent and selective antagonists, xanthines & non-xanthines, have been developed for all the adenosine receptors. A<sub>1</sub> could be considered the best characterized subtype of the adenosine receptor family (Dhalla et al., 2003). The first selective A<sub>1</sub> antagonist DPCPX, which competitively antagonized both the inhibition of AC activity via A<sub>1</sub> and the stimulation via A<sub>2A</sub> with K<sub>i</sub>-values of 0.45 nM at the A<sub>1</sub>AR in rat fat cells, and 330 nM at the A<sub>2A</sub> in human platelets, shows a more than 700-fold A<sub>1</sub>-selectivity (Lohse et al., 1987). DPCPX is a highly selective antagonist for A<sub>1</sub> versus A<sub>2A</sub> (up to 500-fold) and 20-fold versus A<sub>2B</sub> (Johansson et al., 2001). The non-xanthine AR antagonist CGS15943 was the first high affinity A<sub>2A</sub> antagonist described (Francis et al., 1988; Ghai et al., 1987), but it also interacts with appreciable affinity with A<sub>1</sub>, A<sub>2B</sub> & A<sub>3</sub> making it a high affinity non-selective AR antagonist (Kim et al., 1996; Kim et al., 1998). The non-xanthine A<sub>2A</sub> antagonist SCH58261 (Lindstrom et al., 1996) was the first radioligand available for the characterization of the A<sub>2A</sub> in platelets (Dionisotti et al., 1996). SCH58261 has been reported to be 50-fold A<sub>2A</sub>/A<sub>1</sub> selective without appreciable affinity at A<sub>2B</sub> or A<sub>3</sub> (Zocchi et al., 1996). SCH58261 has been reported to be ineffective on HEL cells (A<sub>2B</sub>) up to a concentration of 100 nM, whereas it inhibited the CGS21680 activated cAMP accumulation in HMC-1 cells (A<sub>2A</sub>) (Feoktistov & Biaggioni, 1997). It was also described as a potent and selective A<sub>2A</sub> antagonist in human neutrophil membranes (Varani et al., 1998). Of particular interest for the current body of work, SCH58261 has been described to be useful in the discrimination of A<sub>2B</sub> in cells also expressing A<sub>2A</sub> receptors.

Table 1 Binding affinity of agonists and antagonists at human adenosine receptor subtypes (K<sub>i</sub> values, nM), adapted and modified from (Fredholm et al. 2001)

|          | A <sub>1</sub> AR | A <sub>2A</sub> AR | A <sub>2B</sub> AR | A <sub>3</sub> AR |
|----------|-------------------|--------------------|--------------------|-------------------|
| NECA     | 14                | 20                 | 330                | 6.2               |
| CGS21860 | 290               | 27                 | 361,000            | 67                |
| CGS15943 | 3.5               | 4.2                | 16                 | 51                |
| DPCPX    | 3.9               | 129                | 50                 | 4,000             |
| ZM241385 | 260               | 0.8                | 32                 | >10,000           |
| SCH58261 | 290               | 0.6                | -                  | >10,000           |
| MRS1754  | 400               | 500                | 2                  | 570               |
| MRS1220  | 52                | 11                 | -                  | 0.65              |

Table 2 Summarizes the diverse agonists and antagonists available for identification AR subtypes and their G-protein coupling

|                                | A <sub>1</sub> AR  | A <sub>2A</sub> AR                                    | A <sub>2B</sub> AR   | A <sub>3</sub> AR  |
|--------------------------------|--|---|--|--|
|                                | NECA   |   |  |  |
| universal Selective agonist    | CPA, CHA<br>CCPA, R-PIA  | CGS21860, HE-NECA,<br>CV1808, DPMA, APEC<br>WRC-0470, | None currently available   | 2-CI-IB-MECA   |
| G protein                      | Gi/o   | Gs  | Gs/q/11  | Gi/o/q/11  |
| Effect of G-protein coupling   | ↓ cAMP<br>↑ IP <sub>3</sub> /DAG(PLC)<br>↑ Arachidonate (PLA2)<br>↑ Choline(PLD) | ↑ cAMP<br>↑ ERK1/2                                    | ↑ cAMP<br>↑ IP <sub>3</sub> /DAG<br>↑ ERK1/2<br>↑ [Ca <sup>2+</sup> ] <sub>i</sub> | ↓ cAMP<br>↑ IP <sub>3</sub> /DAG (PLC)                             |
|                                | CGS15943   |   |  |  |
| universal Selective antagonist | DPCPX , N0861<br>8cyclopentyl-theophylline                                       | ZM241385,<br>SCH58261,<br>CSC, KF17387                | MRS1754<br>MRS1706   | MRS1220, F20, RS1067,MRE3008<br>MRS1191,MRS1097<br>MRS1523,L268605 |

Another non-xanthine derivative, ZM241385 (Alexander & Millns, 2001; Kelly et al., 2004) also has high affinity for A<sub>2A</sub> (Poucher et al., 1995), and selectivity, which is up to 1000 fold and 90 fold versus A<sub>1</sub> & A<sub>2B</sub>, respectively, and does not interfere with A<sub>3</sub>AR (Keddie et al., 1996). However, [<sup>3</sup>H]ZM241385 has been reported as a radioligand to label recombinant human A<sub>2B</sub> in HEK293, that do not express A<sub>2A</sub>, with a K<sub>d</sub> value of 33.6 nM (Ji & Jacobson, 1999). It displayed moderate affinity for A<sub>2B</sub> (K<sub>i</sub> 50 nM) in CHO<sub>A2B</sub> cells (Ongini et al., 1999) indicating usefulness as an A<sub>2A</sub>/A<sub>2B</sub> antagonist with moderate A<sub>2A</sub> receptors selectivity. Much progress in this field could be achieved by the development of selective A<sub>2B</sub> antagonists. Because of the low affinity of this receptor for agonists, the design of selective and potent A<sub>2B</sub> antagonists seems to be more promising than the development of selective agonists. Over the last decades, various ADO antagonists have been introduced and modified in the search for A<sub>2B</sub> antagonists. A high affinity A<sub>2B</sub> antagonist is the xanthine derivative MRS1754 considered to be the most potent and selective human A<sub>2B</sub>AR antagonist (Kim et al., 2000). Indeed, the tritiated form [<sup>3</sup>H]-MRS1754 was found to bind specifically to the human A<sub>2B</sub>AR expressed in HEK-293 cells (Ji et al., 2001).

After discovering a significant role for the A<sub>3</sub> in cell cycle regulation and cell growth, generating a receptor antagonist for this subtype became a focus for medicinal chemistry research (Brambilla et al., 2000). A highly potent (0.65 nM) and selective human A<sub>3</sub>AR antagonist MRS1220 was made possible by chemical modification of CGS15943 (Kim et al., 1996). The A<sub>3</sub> has very low affinity (high micromolar range) for the natural AR antagonists, caffeine and theophylline. However, MRS1220 showed affinity (subnanomolar range) at the human A<sub>3</sub> with 80- & 16-fold selectivity versus A<sub>1</sub> and A<sub>2A</sub>, respectively (Baraldi et al., 2000).

## 5. ADENOSINE ACTION & THERAPEUTIC APPLICATIONS

As mentioned previously, the extracellular purine ADO is an important signaling molecule that mediates diverse biological effects, including neurotransmission, exocrine and endocrine secretion, smooth muscle contraction, pain, the immune response, inflammation, platelet aggregation, and modulation of cardiac function, via its receptors (Moro et al., 2006). It is evident that several characteristics of the AR subtypes are, at least in part, elucidated; although more research is necessary to better elucidate the pathophysiological role of these receptors. A series of selective agonists and antagonists are now known, which will greatly enable studies of the roles of these receptors in various diseases. In fact, over the past decade the research in this field has hugely expanded and it seems likely that more roles will be discovered in the future.

A large number of AR agonists and antagonists have been synthesized providing greater or lesser selectivity for

established AR (Table 2). If the selectivity of AR ligands can be improved; it might provide a new approach for treatment of many illnesses, and would also be helpful in limiting possible unwanted effects. Several such compounds are currently undergoing clinical trials for treatment of cardiovascular disorders, pain and diabetic foot ulcer, although the wide distribution and low density of some ARs, or low brain penetration, short half life and lack of effect of these compounds has led to problems. In the cardiovascular system, ADO acting at A<sub>1</sub> is being used successfully as a diagnostic tool and for reversing paroxysmal supraventricular tachycardia (PSVT). There is great attention on the development of A<sub>1</sub> & A<sub>3</sub> agonists for myocardial ischemia (Jacobson & Gao, 2006), while the recently-developed A<sub>2A</sub>agonists Regadenoson (CVT3146) (Hendel et al., 2005), Binodensin (WRC-0470) (Barrett et al., 2005) and ALT-146e are being evaluated in clinical trials for coronary imaging and vasodilation (Jacobson & Gao 2006). The A<sub>1</sub> agonists, GW-493838 (Zambrowicz et al., 2003) and T-62 (Li et al., 2003) have entered clinical trials for treatment of migraine and neuropathic pain, respectively. The A<sub>2A</sub> agonist MRE-0094 is in clinical trials for the treatment of chronic diabetic neuropathic foot ulcers (Jacobson & Gao 2006). In the nervous system, both theophylline and caffeine are well recognized central stimulatory drugs, indeed, most of their stimulant effects are thought to result from antagonism of AR. The role of theophylline and enprofylline in the treatment of asthma and neonatal dyspnea is likely to derive also from adenosine receptor, presumably A<sub>2B</sub>receptor, antagonism (Feoktistov et al., 1998). Employing the antagonistic relationship between striatum dopamine D<sub>2</sub> & A<sub>2A</sub> provides a new approach by using of A<sub>2A</sub> antagonist, such as istradefylline (KW-6002) and V2006 (both in clinical trial), in the treatment of Parkinson's syndrome (Weiss et al., 2003). Another A<sub>1</sub> antagonist in clinical trial is BG9928, which is indicated to improve renal function and congestive heart failure (Auchampach et al., 2004).

## 6. CONCLUSION

Adenosine is an important regulatory metabolite that exerts diverse biological effects in neurons, although the problem in translating this knowledge into therapeutic potential is the nature of adenosine receptors, which widespread in whole body. Even if drugs were developed that could selectively target a receptor mediating a specific action, the problem remains of selectively targeting the receptor at the site of action. However, investigating the factors that could inhibit or induce adenosine receptor gene expression and function, together with regulating the synthesis, release, reuptake or degradation of the purine transmitter, is likely to expand our knowledge of the regulation of adenosine-evoked responses. In summary, it is evident that several characteristics of the AR subtypes are, at least in part, elucidated; although more research is necessary to better

**Neurotransmitter:**  
A chemical in the brain that transmits messages between neurons, or nerve cells.

elucidate the pathophysiological role of these receptors. In fact, over the past decade the research in this field has hugely expanded and it seems likely that more roles will be discovered in the future.

### SUMMARY OF RESEARCH

1. Adenosine receptors (AR) are member of the G-protein Coupled Receptors (GPCR) superfamily, with four subtypes currently recognised, A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> and A<sub>3</sub> receptors
2. The A<sub>2A</sub> and A<sub>2B</sub> both couple via G<sub>s</sub> to adenylcyclase (AC) stimulation (A<sub>2B</sub> can also couple to G<sub>q</sub> subtype), while the A<sub>1</sub> and A<sub>3</sub> are mainly coupled to G<sub>i</sub> protein subtype to G<sub>i</sub> to inhibit AC (although coupling via G<sub>o</sub> and G<sub>q/11</sub> respectively)
3. A<sub>1</sub> and A<sub>2A</sub> are considered high affinity receptors while A<sub>2B</sub> and A<sub>3</sub> receptors are considered as low affinity receptors Because of its potent actions on many organs and systems, adenosine is an obvious target for the development of new drugs
4. AR play a role in multiple physiological functions and further studies necessary to elucidate further their pathophysiological role of these receptors

### FUTURE ISSUES

More Research on Adenosine and Adenosine receptor will help will help understand their pathophysiological roles?

### DISCLOSURE STATEMENT

There is no financial support for this research work from the funding agency.

### ACKNOWLEDGEMENTS

We thank our departments and the Faculty of Medicine, to complete this review for future research

### REFERENCES

1. ABBRACCHIO, M.P., BRAMBILLA, R., CERUTI, S., KIM, H.O., VON LUBITZ, D.K., JACOBSON, K.A. & CATTABENI, F. (1995). G protein-dependent activation of phospholipase C by adenosine A<sub>3</sub> receptors in rat brain. *Mol. Pharmacol.*, 48, 1038-1045.
2. ALEXANDER, S.P. & MILLNS, P.J. (2001). [(3)H]ZM241385--an antagonist radioligand for adenosine A<sub>2A</sub> receptors in rat brain. *Eur. J. Pharmacol.*, 411, 205-210.
3. ALEXANDER, S.P., COOPER, J., SHINE, J. & HILL, S.J. (1996). Characterization of the human brain putative A<sub>2B</sub> adenosine receptor expressed in Chinese hamster ovary (CHO.A2B4) cells. *Br. J. Pharmacol.*, 119, 1286-1290.
4. AUCHAMPACH, J.A., JIN, X., MOORE, J., WAN, T.C., KRECKLER, L.M., GE, Z.D., NARAYANAN, J., WHALLEY, E., KIESMAN, W., TICHON, B., SMITS, G. & GROSS, G.J. (2004). Comparison of three different A<sub>1</sub> adenosine receptor antagonists on infarct size and multiple cycle ischemic preconditioning in anesthetized dogs. *J. Pharmacol. Exp. Ther.*, 308, 846-856.
5. BARALDI, P.G., CACCIARI, B., ROMAGNOLI, R., MERIGHI, S., VARANI, K., BOREA, P.A. & SPALLUTO, G. (2000). A<sub>3</sub> adenosine receptor ligands: history and perspectives. *Med. Res. Rev.*, 20, 103-128.
6. BARRETT, R.J., LAMSON, M.J., JOHNSON, J. & SMITH, W.B. (2005). Pharmacokinetics and safety of binodenoson after intravenous dose escalation in healthy volunteers. *J. Nucl. Cardiol.*, 12, 166-171.
7. BRAMBILLA, R., CATTABENI, F., CERUTI, S., BARBIERI, D., FRANCESCHI, C., KIM, Y.C., JACOBSON, K.A., KLOTZ, K.N., LOHSE, M.J. & ABBRACCHIO, M.P. (2000). Activation of the A<sub>3</sub> adenosine receptor affects cell cycle progression and cell growth. *NaunynSchmiedebergs Arch. Pharmacol.*, 361, 225-234.
8. COOPER, D.M., LONDOS, C. & RODBELL, M. (1980). Adenosine receptor-mediated inhibition of rat cerebral cortical adenylatecyclase by a GTP-dependent process. *Mol. Pharmacol.*, 18, 598-601.
9. DHALLA, A.K., SHRYOCK, J.C., SHREENIWAS, R. & BELARDINELLI, L. (2003). Pharmacology and therapeutic applications of A<sub>1</sub> adenosine receptor ligands. *Curr. Top. Med. Chem.*, 3, 369-385.
10. DICKENSON, J.M., BLANK, J.L. & HILL, S.J. (1998). Human adenosine A<sub>1</sub> receptor and P<sub>2Y2</sub>-purinoceptor-mediated activation of the mitogen-activated protein kinase cascade in transfected CHO cells. *Br. J. Pharmacol.*, 124, 1491-1499.
11. DIONISOTTI, S., FERRARA, S., MOLTA, C., ZOCCHI, C. & ONGINI, E. (1996). Labeling of A<sub>2A</sub> adenosine receptors in human platelets by use of the new nonxanthine antagonist radioligand [3H]SCH 58261. *J. Pharmacol. Exp. Ther.*, 278, 1209-1214.
12. FEOKTISTOV, I. & BIAGGIONI, I. (1997). Adenosine A<sub>2B</sub> receptors. *Pharmacol. Rev.*, 49, 381-402
13. FEOKTISTOV, I., POLOSA, R., HOGATE, S.T. & BIAGGIONI, I. (1998). Adenosine A<sub>2B</sub> receptors: a novel therapeutic target in asthma? *Trends Pharmacol. Sci.*, 19, 148-153.
14. FRANCIS, J.E., CASH, W.D., PSYCHOYOS, S., GHAI, G., WENK, P., FRIEDMANN, R.C., ATKINS, C., WARREN, V., FURNESS, P., HYUN, J.L. & . (1988). Structure-activity profile of a series of novel triazoloquinazoline adenosine antagonists. *J. Med. Chem.*, 31, 1014-1020.
15. FREDHOLM, B.B., ABBRACCHIO, M.P., BURNSTOCK, G., DALY, J.W., HARDEN, T.K., JACOBSON, K.A., LEFF, P. & WILLIAMS, M. (1994). Nomenclature and classification of purinoceptors. *Pharmacol. Rev.*, 46, 143-156.
16. FREDHOLM, B.B., ARSLAN, G., HALLDNER, L., KULL, B., SCHULTE, G. & WASSERMAN, W. (2000). Structure and function of adenosine receptors and their genes. *NaunynSchmiedebergs Arch. Pharmacol.*, 362, 364-374.
17. FURLONG, T.J., PIERCE, K.D., SELBIE, L.A. & SHINE, J. (1992). Molecular characterization of a human brain adenosine A<sub>2</sub> receptor. *Brain Res. Mol. Brain Res.*, 15, 62-66.
18. GAO, Z., CHEN, T., WEBER, M.J. & LINDEN, J. (1999). A<sub>2B</sub> adenosine and P<sub>2Y2</sub> receptors stimulate mitogen-activated protein kinase in human embryonic kidney-293 cells. cross-talk between cyclic AMP and protein kinase c pathways. *J. Biol. Chem.*, 274, 5972-5980.
19. GHAI, G., FRANCIS, J.E., WILLIAMS, M., DOTSON, R.A., HOPKINS, M.F., COTE, D.T., GOODMAN, F.R. & ZIMMERMAN, M.B. (1987). Pharmacological characterization of CGS 15943A: a novel nonxanthine adenosine antagonist. *J. Pharmacol. Exp. Ther.*, 242, 784-790
20. GRAHAM, S., COMBES, P., CRUMIERE, M., KLOTZ, K.N. & DICKENSON, J.M. (2001). Regulation of p42/p44 mitogen-activated protein kinase by the human adenosine A<sub>3</sub> receptor in transfected CHO cells. *Eur. J. Pharmacol.*, 420, 19-26.
21. ITO, H., VEREECKE, J. & CARMELIET, E. (1994). Mode of regulation by G protein of the ATP-sensitive K<sup>+</sup> channel in guinea-pig ventricular cell membrane. *J. Physiol.*, 478 ( Pt 1), 101-107.
22. JACOBSON, K.A. & GAO, Z.G. (2006). Adenosine receptors as therapeutic targets. *Nat. Rev. Drug Discov.*, 5, 247-264.
23. JACOBSON, K.A. & GAO, Z.G. (2006). Adenosine receptors as therapeutic targets. *Nat. Rev. Drug Discov.*, 5, 247-264.
24. JACOBSON, K.A., VAN GALEN, P.J. & WILLIAMS, M. (1992). Adenosine receptors: pharmacology, structure-activity relationships, and therapeutic potential. *J. Med. Chem.*, 35, 407-422.
25. JARVIS, M.F., SCHULZ, R., HUTCHISON, A.J., DO, U.H., SILLS, M.A. & WILLIAMS, M. (1989). [3H]CGS 21680, a selective A<sub>2</sub> adenosine receptor agonist directly labels A<sub>2</sub> receptors in rat brain. *J. Pharmacol. Exp. Ther.*, 251, 888-893.
26. JI, X., KIM, Y.C., AHERN, D.G., LINDEN, J. & JACOBSON, K.A. (2001). [3H]MRS 1754, a selective antagonist radioligand for A<sub>2B</sub> adenosine receptors. *Biochem. Pharmacol.*, 61, 657-663.
27. JI, X.D. & JACOBSON, K.A. (1999). Use of the triazoloquinazoline [3H]ZM 241385 as a radioligand at recombinant human A<sub>2B</sub> adenosine receptors. *Drug Des Discov.*, 16, 217-226

**Dhalla et al. (2003):** Adenosine's diverse physiological functions are mediated by four subtypes of receptors (A(1), A(2A), A(2B) and A(3)). The A(1) adenosine receptor pharmacology and therapeutic application of ligands for this receptor are the subjects of this review. A(1) receptors are present on the surface of cells in organs throughout the body. Actions mediated by A(1) receptors include slowing of heart rate and AV nodal conduction, reduction of atrial contractility, attenuation of the stimulatory actions of catecholamines on beta-adrenergic receptors, reduction of lipolysis in adipose tissue, reduction of urine formation, and inhibition of neuronal activity. Although adenosine analogs with high efficacy, affinity, and selectivity for the A(1) receptor are available, the ubiquitous distribution and wide range of physiological actions mediated by A(1) receptors are obstacles to development of therapeutic agents that activate these receptors. However, it may be possible to exploit the high A(1) "receptor reserve" for some actions of adenosine by use of weak (partial) agonists to target these actions while avoiding others for which receptor reserve is low. The presence of high receptor reserves for the anti-arrhythmic and anti-lipolytic actions of adenosine suggests that partial A(1) agonists could be used as anti-arrhythmic and anti-lipolytic agents. In addition, allosteric enhancers of the binding of adenosine to A(1) receptors could be used therapeutically to potentiate desirable effects of endogenous adenosine. Antagonists of the A(1) receptor can increase urine formation, and because they do not decrease renal blood flow, are particularly useful to maintain glomerular filtration in patients having edema secondary to reduced cardiac function.

28. JOHANSSON,B., GEORGIEV,V., PARKINSON,F.E. & FREDHOLM,B.B. (1993). The binding of the adenosine A2 receptor selective agonist [3H]CGS 21680 to rat cortex differs from its binding to rat striatum. *Eur. J. Pharmacol.*, 247, 103-110.
29. JOHANSSON,B., HALLDNER,L., DUNWIDDIE,T.V., et al.(2001). Hyperalgesia, anxiety, and decreased hypoxic neuroprotection in mice lacking the adenosine A1 receptor. *Proc. Natl. Acad. Sci. U. S. A*, 98, 9407-9412.
30. KAPLAN,I.V., KAPLAN,A.V. & FISHER,J.D. (2000). Adenosine induced atrial fibrillation precipitating polymorphic ventricular tachycardia. *Pacing Clin. Electrophysiol.*,23, 140-141.
31. KEDDIE,J.R., POUCHER,S.M., SHAW,G.R., BROOKS,R. & COLLIS,M.G. (1996). In vivo characterisation of ZM 241385, a selective adenosine A2A receptor antagonist. *Eur. J Pharmacol.*, 301, 107-113
32. KELLY,M., BAILEY,A., LEDENT,C., KITCHEN,I. & HOURANI,S. (2004). Characterization of [3H]ZM 241385 binding in wild-type and adenosine A2A receptor knockout mice. *Eur. J Pharmacol.*,504, 55-59.
33. KIM,Y.C., DE,Z.M., CHANG,L., MORO,S., VON FRIJTAG DRABBE KUNZEL JK, MELMAN,N., IJZERMAN,A.P. & JACOBSON,K.A. (1998). Derivatives of the triazoloquinazoline adenosine antagonist (CGS 15943) having high potency at the human A2B and A3 receptor subtypes. *J Med. Chem.*, 41, 2835-2845
34. KIM,Y.C., JI,X., MELMAN,N., LINDEN,J. & JACOBSON,K.A. (2000). Anilide derivatives of an 8-phenylxanthine carboxylic congener are highly potent and selective antagonists at human A(2B) adenosine receptors. *J. Med. Chem.*, 43, 1165-1172.
35. KIM,Y.C., JI,X.D. & JACOBSON,K.A. (1996). Derivatives of the triazoloquinazoline adenosine antagonist (CGS15943) are selective for the human A3 receptor subtype. *J Med. Chem.*, 39, 4142-4148.
36. KLOTZ,K.N., HESSLING,J., HEGLER,J., OWMAN,C., KULL,B., FREDHOLM,B.B. & LOHSE,M.J. (1998). Comparative pharmacology of human adenosine receptor subtypes - characterization of stably transfected receptors in CHO cells. *NaunynSchmiedebergs Arch. Pharmacol.*, 357, 1-9.
37. LI,X., CONKLIN,D., PAN,H.L. & EISENACH,J.C. (2003). Allosteric adenosine receptor modulation reduces hypersensitivity following peripheral inflammation by a central mechanism. *J Pharmacol. Exp. Ther.*, 305, 950-955.
38. LINDEN,J., THAI,T., FGLER,H., JIN,X. & ROBEVA,A.S. (1999). Characterization of human A(2B) adenosine receptors: radioligand binding, western blotting, and coupling to G(q) in human embryonic kidney 293 cells and HMC-1 mast cells. *Mol. Pharmacol.*, 56, 705-713.
39. LINDSTROM,K., ONGINI,E. & FREDHOLM,B.B. (1996). The selective adenosine A2A receptor antagonist SCH 58261 discriminates between two different binding sites for [3H]-CGS 21680 in the rat brain. *NaunynSchmiedebergs Arch. Pharmacol.*, 354, 539-541.
40. LOHSE,M.J., KLOTZ,K.N., LINDENBORN-FOTINOS,J., REDDINGTON,M., SCHWABE,U. & OLSSON,R.A. (1987). 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX)--a selective high affinity antagonist radioligand for A1 adenosine receptors. *NaunynSchmiedebergs Arch. Pharmacol.*, 336, 204-210.
41. MATHERNE,G.P., LINDEN,J., BYFORD,A.M., GAUTHIER,N.S. & HEADRICK,J.P. (1997). Transgenic A1 adenosine receptor overexpression increases myocardial resistance to ischemia. *Proc. Natl. Acad. Sci. U. S. A*, 94, 6541-6546.
42. MEGSON,A.C., DICKENSON,J.M., TOWNSEND-NICHOLSON,A. & HILL,S.J. (1995). Synergy between the inositol phosphate responses to transfected human adenosine A1-receptors and constitutive P2-purinoreceptors in CHO-K1 cells. *Br. J. Pharmacol.*, 115, 1415-1424.
43. MIRABET,M., HERRERA,C., CORDERO,O.J., MALLOL,J., LLUIS,C. & FRANCO,R. (1999). Expression of A2B adenosine receptors in human lymphocytes: their role in T cell activation. *J Cell Sci.*, 112 ( Pt 4), 491-502.
44. MITCHELL,C.H., PETERSON-YANTORNO,K., CARRE,D.A., MCGLINN,A.M., COCA-PRADOS,M., STONE,R.A. & CIVAN,M.M. (1999). A3 adenosine receptors regulate Cl- channels of nonpigmented ciliary epithelial cells. *Am. J Physiol.* 276, C659-C666
45. MORO,S., GAO,Z.G., JACOBSON,K.A. & SPALLUTO,G. (2006). Progress in the pursuit of therapeutic adenosine receptor antagonists. *Med. Res. Rev.*, 26, 131-159.
46. NEVES,S.R., RAM,P.T. & IYENGAR,R. (2002). G protein pathways. *Science*, 296, 1636-1639.HAMM,H.E. (1998). The many faces of G protein signaling. *J Biol. Chem.*, 273, 669-672
47. OLAH,M.E. & STILES,G.L. (1995). Adenosine receptor subtypes: characterization and therapeutic regulation. *Annu. Rev. Pharmacol. Toxicol.*, 35, 581-606.
48. OLAH,M.E. (1997). Identification of A2a adenosine receptor domains involved in selective coupling to Gs. Analysis of chimeric A1/A2a adenosine receptors. *J Biol. Chem.*, 272, 337-344.
49. ONGINI,E., DIONISOTTI,S., GESSI,S., IRENIUS,E. & FREDHOLM,B.B. (1999). Comparison of CGS 15943, ZM 241385 and SCH 58261 as antagonists at human adenosine receptors. *NaunynSchmiedebergs Arch. Pharmacol.*, 359, 7-10.
50. OU,Q.Y., LI,C., OLAH,M.E., JOHNSON,R.A., STILES,G.L. & CIVELLI,O. (1992). Molecular cloning and characterization of an adenosine receptor: the A3 adenosine receptor. *Proc. Natl. Acad. Sci. U. S. A*, 89, 7432-7436.
51. PALMER,T.M., GETTYS,T.W. & STILES,G.L. (1995). Differential interaction with and regulation of multiple G-proteins by the rat A3 adenosine receptor. *J Biol. Chem.*, 270, 16895-16902.
52. POUCHER,S.M., KEDDIE,J.R., SINGH,P., STOGGALL,S.M., CAULKETT,P.W., JONES,G. & COLL,M.G. (1995). The in vitro pharmacology of ZM 241385, a potent, non-xanthine A2a selective adenosine receptor antagonist. *Br. J Pharmacol.*, 115, 1096-1102.
53. RANG,H.P., DALE,M.M., RITTER,J.M. & MOORE,P.K. (2003). *Pharmacology* Churchill Livingstone, Scotland, UK
54. ROBINSON,A.J. & DICKENSON,J.M. (2001). Regulation of p42/p44 MAPK and p38 MAPK by the adenosine A(1) receptor in DDT(1)MF-2 cells. *Eur. J Pharmacol.*, 413, 151-161
55. SCHULTE,G. & FREDHOLM,B.B. (2000). Human adenosine A(1), A(2A), A(2B), and A(3) receptors expressed in Chinese hamster ovary cells all mediate the phosphorylation of extracellular-regulated kinase 1/2. *Mol. Pharmacol.*, 58, 477-482.
56. SEGER,R. & KREBS,E.G. (1995). The MAPK signaling cascade. *FASEB J*, 9, 726-735.
57. SEIDEL,M.G., KLINGER,M., FREISSMUTH,M. & HOLLER,C. (1999). Activation of mitogen-activated protein kinase by the A(2A)-adenosine receptor via a rap1-dependent and via a p21(ras)-dependent pathway. *J Biol. Chem.*, 274, 25833-25841.
58. SELX,V., MANCUSI,G., HOLLER,C., GLORIA-MAERCKER,E., SCHUTZ,W. & FREISSMUTH,M. (1997). Stimulation of the mitogen-activated protein kinase via the A2A-adenosine receptor in primary human endothelial cells. *J. Biol. Chem.*, 272, 5792-5799.
59. STEHLE,J.H., RIVKEES,S.A., LEE,J.J., WEAVER,D.R., DEEDS,J.D. & REPPERT,S.M. (1992). Molecular cloning and expression of the cDNA for a novel A2-adenosine receptor subtype. *Mol. Endocrinol.*, 6, 384-393.
60. SWEENEY,M.I. & DOLPHIN,A.C. (1995). Adenosine A1 agonists and the Ca2+ channel agonist bay K 8644 produce a synergistic stimulation of the GTPase activity of Go in rat frontal cortical membranes. *J. Neurochem.*, 64, 2034-2042
61. TENG,B., QIN,W., ANSARI,H.R. & MUSTAFA,S.J. (2005). Involvement of p38-mitogen-activated protein kinase in adenosine receptor-mediated relaxation of coronary artery. *Am. J Physiol Heart Circ. Physiol.* 288, H2574-H2580.
62. VARANI,K., GESSI,S., DIONISOTTI,S., ONGINI,E. & BOREA,P.A. (1998). [3H]-SCH 58261 labelling of functional A2A adenosine receptors in human neutrophil membranes. *Br. J Pharmacol.*, 123, 1723-1731.
63. WEISS,S.M., BENWELL,K., CLIFFE,I.A., GILLESPIE,R.J., KNIGHT,A.R., LERPINIÈRE,J., MISRA,A., PRATT,R.M., REVELL,D., UPTON,R. & DOURISH,C.T. (2003). Discovery of nonxanthine adenosine A2A receptor antagonists for the treatment of Parkinson's disease. *Neurology*, 61, S101-S106.
64. YOSHIMURA,Y., KRISTO,G., KEITH,B.J., JAHANIA,S.A., MENTZER,R.M., JR. & LASLEY,R.D. (2004). The p38 MAPK inhibitor SB203580 blocks adenosine A(1) receptor-induced attenuation of in vivo myocardial stunning. *Cardiovasc. Drugs Ther.*,18, 433-440.
65. ZAMBROWICZ,B.P., TURNER,C.A. & SANDS,A.T. (2003). Predicting drug efficacy: knockouts model pipeline drugs of the pharmaceutical industry. *Curr. Opin. Pharmacol.*,3, 563-570.

66. ZOCCHI,C., ONGINI,E., CONTI,A., MONOPOLI,A., NEGRETTI,A., BARALDI,P.G. & DIONISOTTI,S. (1996). The non-xanthine heterocyclic compound SCH 58261 is a new potent and selective A2a adenosine receptor antagonist. *J Pharmacol. Exp. Ther.*, 276, 398-404.

## RELATED RESOURCE

1. Marinissen, M. J. and Gutkind, J. S. (2001). G-protein-coupled receptors and signaling networks: emerging paradigms. *Trends Pharmacol. Sci.* 22, 368-375.
2. Venter, J. C., Adams, M. D., Myers, E. W., Li, P. W., Mural, R. J., Sutton, G. G., Smith, H. O., Yandell, M., Evans, C. A., Holt, R. A. et al. (2001). The sequence of the human genome. *Science* 291, 1304-1351.