INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com Volume-4, Issue-3, July-Sept-2013 Coden : IJABPT Copyrights@2013

ISSN : 0976-4550

Received: 05th Oct-2012 *Revised: 18th Dec-2012* *Accepted: 20th May-2013*

Research Article

A_{2B} ADENOSINE RECEPTORS IN SH-SY5Y CELLS-FUNCTIONAL APPROACHES

Nouh M.H. Aljarari[1*], Steve S. PH. Alexander[2] and D.S.Sheriff

[1]Department of Pharmacology and Biochemistry, Faculty of Medicine, Benghazi University, Benghazi, Libya

[2]School of Biomedical Sciences, Faculty of Medicine, University of Nottingham, UK

ABSTRACT: The diversity in intracellular signalling downstream of adenosine receptors is dependent on the receptor subtype activated by adenosine. A_1 and A_{2A} are considered high affinity receptors while A_{2B} and A_3 are considered as low affinity receptors. Despite the apparent widespread distribution of A_{2B} in every cell of every species, the receptor number and low affinity for adenosine and its analogue, makes it the least well-characterised adenosine receptor. A_{2B} adenosine receptors are classically coupled to G_s protein and elevation of cAMP levels in target cells, although, A_{2B} have also been known to couple to G_q and not just G_s proteins, and also to couple to Mitogen activated protein kinases (MAPK). In the present study, changes in intracellular calcium concentration ($[Ca^{2+}]_i$) and MAPK phosphorylation following subtype-specific and non-specific agonists and antagonists stimulation were investigated in human neuroblastoma SH-SY5Y cell line. In conclusion, our results indicate that SH-SY5Y cells express A_{2B} adenosine receptors that are coupled to stimulation of Extracellular regulated kinase (ERK1/2) mitogen activated protein (MAP) kinases.

Key words: Adenosine receptors, A_{2B}, MAPK, ERK1/2, SH-SY5Y and human neuroblastoma.

INTRODUCTION

Adenosine (ADO) produces a wide variety of effects throughout the body via activation of specific cell surface receptors, known as adenosine receptors (AR). Functional and molecular studies made it possible to classify adenosine receptors as A_1, A_{2A}, A_{2B} and A_3 by the NC-IUPHAR committee on Receptor Nomenclature and Drug classification((Alexander, Mathie, Peters 2008). Under physiological conditions ADO is shown to be present extracellularly at concentrations that can stimulate both the higher affinity A_1 and A_{2A}, while under pathological conditions, ADO rises to concentrations that can also stimulate the lower affinity A_{2B} and A_3(Bruns, Lu, Pugsley1986). It seems that A_{2B} and A_3 are much more active in pathophysiological events when extracellular ADO concentration rises above the normal concentrations (Ralevic,Burnstock,1997). Depending on the subtype, the activation of the G protein gives rise to different signalling events. Although the accepted signal cascade of both A_{2A}(Olah 1997) and A_{2B}(Olah , Stiles 1995) is to increase cAMP generation via a positive coupling to AC by G_s protein, the activation of PLC with stimulation of IP_3 levels and calcium elevation has been observed to be a further signal transduction pathway by which the A_{2B} may evoke the cellular response. For instance, NECA was found to stimulate an increase in $[Ca^{2+}]_i$ and cAMP accumulation in the human mast cell HMC-1 cells (Linden et.al 1999). In human erythroleukaemia (HEL) cells, activation of A_{2B} evoked a Ca^{2+} influx, (Feoktistov,Murray,Biaggioni 1994). In contrast, in human T lymphocytes, A_{2B} activation by NECA results in cAMP generation, with no alteration in $[Ca^{2+}]_i$(Mirabet et al,1999).

In addition, there is increasing evidence to demonstrate that it is not the only second messenger pathways that can activated by these subtypes (Fredholm et al 2000). For instance, all the human AR transfected into Chinese hamster ovary (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).The diverse effects of ADO on mitogenesis, depending on the receptor subtype activated, may be related to changes in the mitogen-activated protein kinases (MAPK) pathway. The MAPK is used for trans-cytoplasmic signalling to the nucleus, where transcription of specific genes is induced through phosphorylation and activation of transcription factors, leading to changes in gene expression profiles and to promote differentiation (Hunter,1995,Hancock,2005). There are three distinct MAP kinase signal transduction pathways, include the extracellular signal-regulated kinases, ERK1/2 (also known as p42/44 MAPKs), the c-Jun N-terminal kinases/stress-activated protein kinases (JNK/SAPK) and the p38 kinases (p38) (Miloso et al.2004).

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

Activation of the ERK1/2 cascade pathway via AR has been intensively studied in a variety of systems. For example, A_1 stimulation has also been shown to activate both ERK1/2 and p38 phosphorylation in DDT1MF-2 cells (Robinson , Dickenson 2001), and p38 in pigmyocardial stunning (Yoshimura et al.2004).The selective A_1 agonist N^6-cyclopentyladenosine (CPA) has also been reported to increase MAP kinase activity in CHO tranfected with A_1 (CHO-A_1) cells (Dickenson, Blank , Hill , 1998). CGS21680 (A_{2A} selective agonist) has also been reported to stimulate MAP kinase activity in HEK293 and CHO cells transfected with A_{2A}(Seidel et al.1999). The stimulation of endogenous A_{2B} by NECA has also been suggested to evoke activation of ERK1/2 in HEK293 cells (Gao et al.1999), and in HMC-1 (Feoktistov, Goldstein, Biaggioni 1999). CHO with A_3 (CHO-A_3) cells have also been reported to produce concentration-dependent increases in ERK1/2 MAPK in response to IB-MECA, an A_3 high affinity agonist (Graham et al.2001). In human endothelial cells, MAPK pathway have been shown to be activated by A_{2A} through a tyrosine kinase, which appears to play an important role in cell differentiation, proliferation and death (Sexl et al.1997). Stimulation of A_{2B} via NECA has been reported to regulate the ERK, JNK and p38 MAPK signalling cascades in HMC-1 cells (Feoktistov, Goldstein , Biaggioni,1999) and p38 MAPK phosphorylation in porcine coronary smooth muscle (Teng et al.2005).

MATERIALS & METHODS

SH-SY5Y, cells were from were purchased from the European Collection of Cell Culture, UK**.** acrylamide, bis-acrylamide mixture was obtained from Geneflow, UK. Enhanced chemiluminescent detection reagent was obtained from Amersham Bioscience, UK. FLUO-4 and pluronic acid β-Actin were purchased from Invitrogen, while carbachol, probenecid, Brilliant Black and HBSS supplemented were purchased from Sigma. All primary antibodies were raised in rabbit; the only exceptions were β-actin was raised in mouse. Phospho-ERK1/2 & ERK1/2 MAPK, Phospho-p38& p38 MAP, Phospho- JNK & JNK were from Cell Signalling.

SH-SY5Y cells were cultured in vitro and maintained in medium consisting of a mixture of nutrient mixture Ham's F-12 and Eagle's minimal essential medium supplemented with heat inactivated FBS, L-glutamine, non-essential amino acid solution and penicillin/streptomycin. For passaging, the pellet was obtained by trypsinization with fresh medium being replaced. Cell counting was done using the Trypan blue inclusion and exclusion method.

Measurement of changes in $[Ca^{2+}]_i$ concentration: Confluent SH-SY5Y cells, growing as monolayers in 96 well black-walled plates, were loaded with FLUO-4 (pluronic acid in DMSO) in a loading buffer (probenecid & Brilliant Black) The antagonist was added with loading buffer, where indicated. After the dye incubation period, the plate was loaded on the FlexStation and analysed using FLUO protocol. Measuring of fluorescence in each well was conducted up to 500 seconds with addition of carbachol and NECA at 20 and 300 seconds, respectively**.**

MAPK activation assays: the 90% confluence SH-SY5Y cells were rendered quiescent by serum starvation 24 hours prior to MAPK phosphorylation assays. On the experimental day, an additional period of incubation in fresh serum-free medium was performed to minimize basal activity. Cells were incubated in the presence of antagonists, where indicated. Cells were then stimulated by addition of 15% FBS or adenosine receptor agonist for a further incubation period. Reactions were terminated by rapidly removing the medium and placing the cells on ice followed by twice rinsing with ice-cold Phosphate buffered saline (PBS). The cells were then harvested and the total protein was extracted and the immunoreactivity studies were performed as described below.

Total protein extraction: The cells were harvested using non-enzymatic cell dissociation solution (Sigma, UK). The detached cells were centrifuged and the cell pellet was lyzed with lysis buffer. The cell homogenate was obtained by disrupting the cells with a Polytron homogenizer. A Triton-insoluble fraction was separated by centrifugation. Protein concentrations were determined via a micro-assay described by Lowry et al. (1951), the correct protein concentration was calculated by using the Revelation software. Electrophoresis and Western blotting: protein samples were diluted in solubilising buffer. The Triton-soluble fractions were separated on 10% SDS-polyacrylamide gels using a Bio-Rad Mini-PROTEAN® 3 Electrophoresis apparatus (Bio-Rad, UK) and transferred to nitrocellulose membrane. The MAPK activation was assayed by incubating nitrocellulose blots, overnight at particular dilutions, with an antiserum that recognizes only the phosphorylated (active) forms of ERK1/2 MAP kinase, p38, and JNK. In order to rule out that the differences observed were due to the application of unequal amounts of lysates, control blots were also probed with an antiserum recognizing the unphosphorylated (inactive) form.

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

Visualizing the protein bands were detected based on the principle of enhanced chemiluminescence detection system (Amersham Biosciences). The developed Western blot films were scanned with an image densitometer (Bio-Rad, UK), Densitometric analysis and molecular weight were measured and analysed using Quantity One software (Bio-Rad, UK). Data are presented as means ± standard error of the mean (S.E.M) of at least three independent experiments. Data were analysed for statistically significant differences using Prism 4 software (Graphpad, USA).

RESULTS

Measurement of Changes in $[Ca^{2+}]_i$ elicited by NECA

Given that A_{2B} expressed endogenously in HEK293 cells evoke an increase in $[Ca^{2+}]_i$ following pre-stimulation with carbachol (Jackson, J. HSJ, Alexander 2003), a similar mode of coupling was investigated in SH-SY5Y cells (Figure 1). Although carbachol evoked a clear elevation in $[Ca^{2+}]_i$, NECA failed to alter $[Ca^{2+}]_i$ in the absence or presence of muscarinic receptor pre-stimulation.

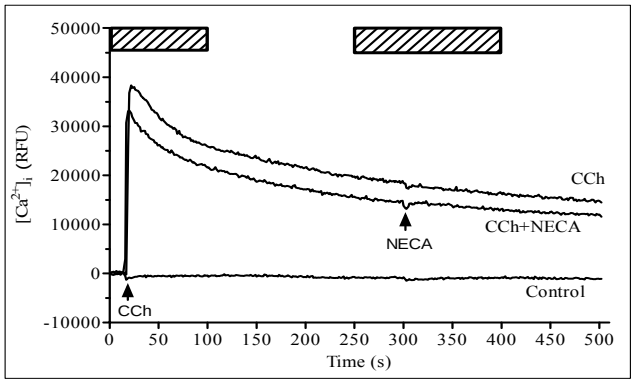

Figure 1 Changes in intracellular calcium in SH-SY5Y cells. Indicated are responses in the absence (control) and presence of 1 µM carbachol (CCh) added at 20s. Also included is a trace for additions 1 µM carbachol followed by 10 µM NECA at 300s (CCh+NECA). Data are means from a single experiment conducted in triplicate. Boxes above the traces indicate time bins for data collection of changes in fluorescence used in the subsequent concentration-response analysis.

The quantitative analysis of the effects of carbachol and AR ligands on $[Ca^{2+}]_i$ in SH-SY5Y cells. Carbachol evoked a clear elevation in $[Ca^{2+}]_i$ in the early monitoring period (up to 100 s), which was not different in the wells which would later receive NECA or which had been pre-incubated in the presence of SCH58261 (A_{2A} antagonist) or MRS1754 (A_{2B} antagonist) (Figure 2A). During the second monitoring period (250-400 s), (Figure 1 & 2B), $[Ca^{2+}]_i$ was still elevated above basal in the presence of carbachol, but not altered further following NECA application in the absence or presence of AR antagonists.

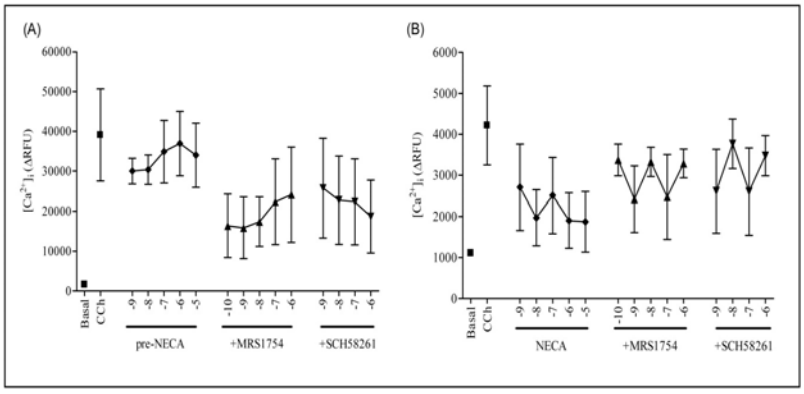

Figure 2 Changes in intracellular calcium in SH-SY5Y cells.

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

Panel A shows the maximal change (peak-trough) in relative fluorescence units (RFU) over the period 1-100 s, in the absence (basal) or presence of 1 µM carbachol. Some wells of the microtitre plate were pre-incubated in the presence of the indicated concentrations of MRS1754 or SCH58261, prior to carbachol addition. Also delineated are carbachol-evoked calcium responses in cells which later receive the indicated concentrations of NECA.

Panel B shows the maximal change (peak-trough) in relative fluorescence units (RFU) over the period 250-400 s, in the absence (basal) or presence of 1 µM carbachol. Some wells of the microtitre plate were pre-incubated in the presence of the indicated concentrations of MRS1754 or SCH58261, prior to carbachol addition. Also delineated are carbachol-evoked calcium responses in cells which receive the indicated concentrations of NECA during the data collection period. Data are means ± SEM from single experiment conducted in triplicate and was repeated in a further occasion with lack of calcium response.

The next step in investigation the A_{2B} signalling pathway, after the NECA failed to change $[Ca^{2+}]_i$ in the absence or presence of muscarinic receptor pre-stimulation, is to examine activation of MAPK phosphorylation.

Activation of MAPK pathway by AR in SH-SY5Y cells

In cells expressing more than one AR, selective antagonists provide an alternative approach when used in conjunction with the non-selective agonist NECA to allow selective stimulation of particular subtypes. Only non-selective agonists are available to investigate the functional expression of A_{2B} and so results obtained from using this approach are difficult to interpret in cells also expressing A_{2A}. Since the non-selective agonists will activate both subtypes, it is impossible to exclude the possibility of A_{2A} to modulate responses. CGS21680 has proven particularly useful, since it is one of the most potent agonists at A_{2A} but is virtually ineffective at A_{2B} in PC12 cells (Hide et al.1992).

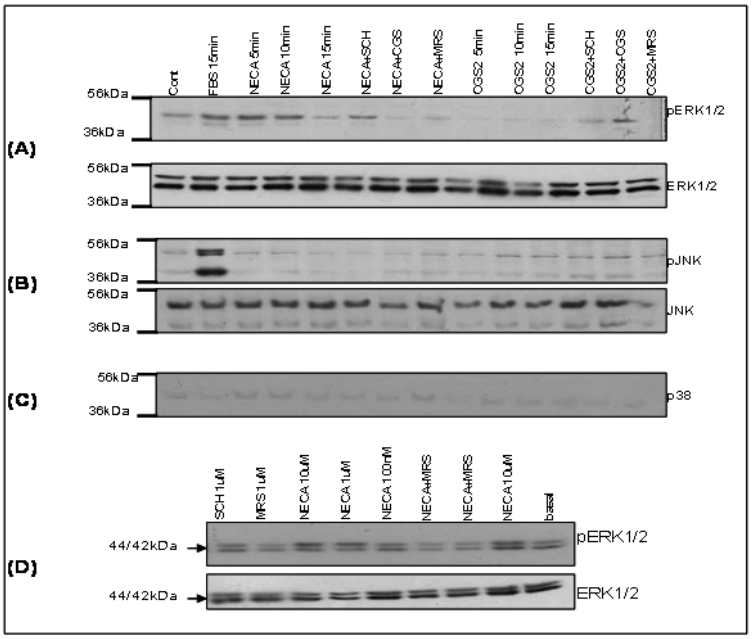

Figure 3 Immunoblot of SH-SY5Y cells probed with MAPK antibodies. **(A)** NECA & CGS21680-induced ERK-1/2 phosphorylation. Cells were treated for increasing time periods (5, 10 & 15 min) with either 10 µM NECA or CGS21680 (CGS2) Antagonists (SCH, SCH58261; CGS, CGS15943; MRS, MRS1754) were added to a final concentration of 1 µM 15 min prior to agonist addition. (B) &(C) Similar to (A) but with JNK & p38 antibodies. (D) Cells were treated with increasing concentrations of NECA (100 nM-10 µM, for 5 min) in the presence of 1 µM SCH 58261, and AR antagonists alone (1 µM of MRS1754 or SCH58261, for 15 min). p refer to the phosphorylated MAPK and the blot without p represent the total MAPK. Data are representative of a single experiment, repeated on two further occasions.

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

Of the three enzymes, which are the principal MAP kinase isoforms (ERK1/2, JNK and p38 MAPK), it was only possible to detect changes in ERK1/2, but not JNK or p38, in response to AR activation (Figure 3). Time course experiments revealed that, in SH-SY5Y cells, a peak of activity was observed at 5 minutes with 10 µM NECA, with no response to 10 µM CGS21680 (Figure 3 and 4). The ERK-1/2 phosphorylation induced by NECA was inhibited by the selective A_{2B} antagonist MRS1754, but not SCH58261 (Figure 5). NECA evoked a concentration-dependent ERK1/2 phosphorylation in the presence of the A_{2A} antagonist SCH58261 (Figure 6).

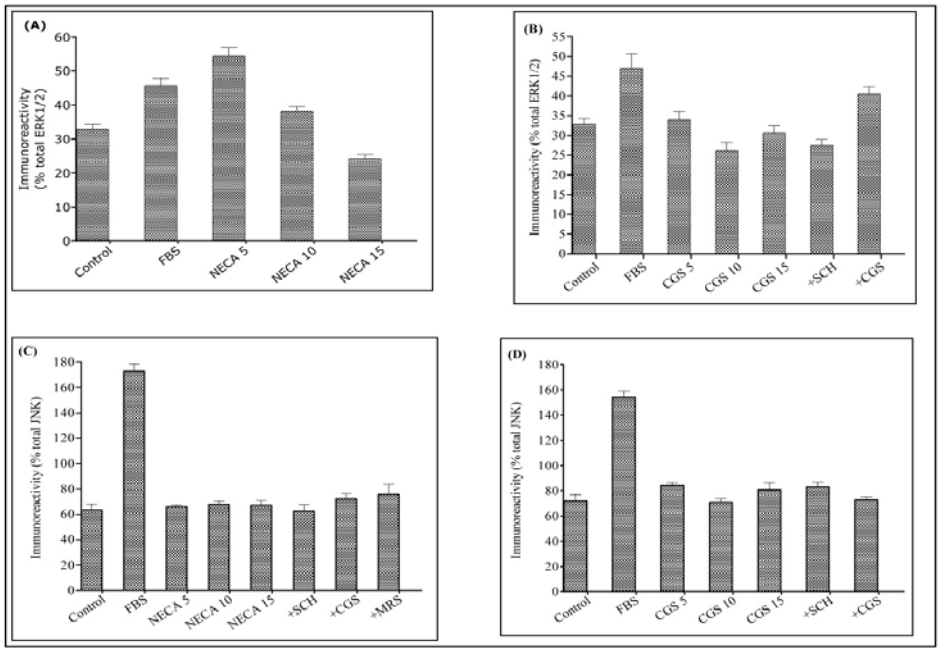

Figure 4 Quantification of phosphoMAPK bands, from immunoblots of SH-SYSY cells stimulated with 10 µM NECA or CGS21680 in the presence and absence of AR antagonists for the indicated time period [(5, 10 ,15 min); (pERK1/2, A & B) or (pJNK C & D)]. Antagonists (SCH, SCH58261; CGS, CGS15943; MRS, MRS1754) were added to a final concentration of 1 µM. Results are expressed as a percentage of either total ERK1/2 or JNK; data are means ± SEM of three independent experiments.

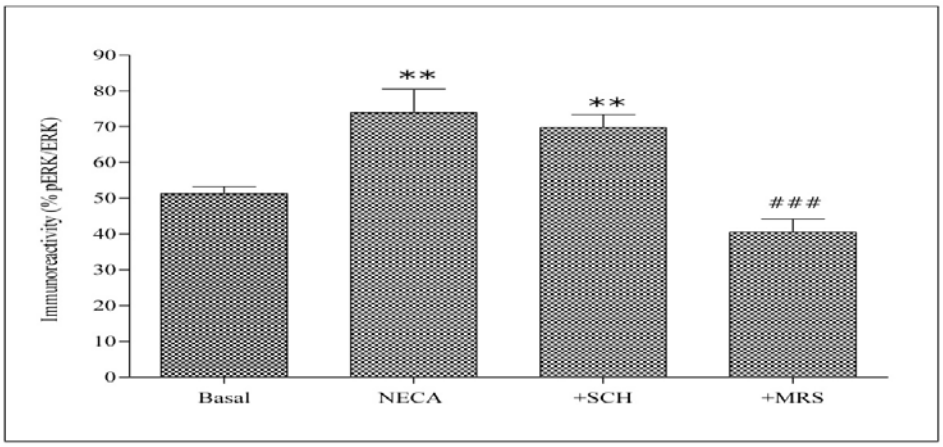

Figure 5 Quantification of phosphoMAPK bands, from immunoblots of SH-SYSY cells stimulated with 10 µM NECA for 5 min, in the presence and absence of AR antagonists. Antagonists (SCH, SCH58261; MRS, MRS1754) were added to a final concentration of 1 µM. Results are expressed as a percentage of total ERK1/2; data are means ± SEM of three independent experiments. **P<0.01 compared to basal, ###P<0.001 presence vs. absence of antagonist. Data were analysed for statistical significance using one way ANOVA with Bonferonni's Multiple Comparison test.

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

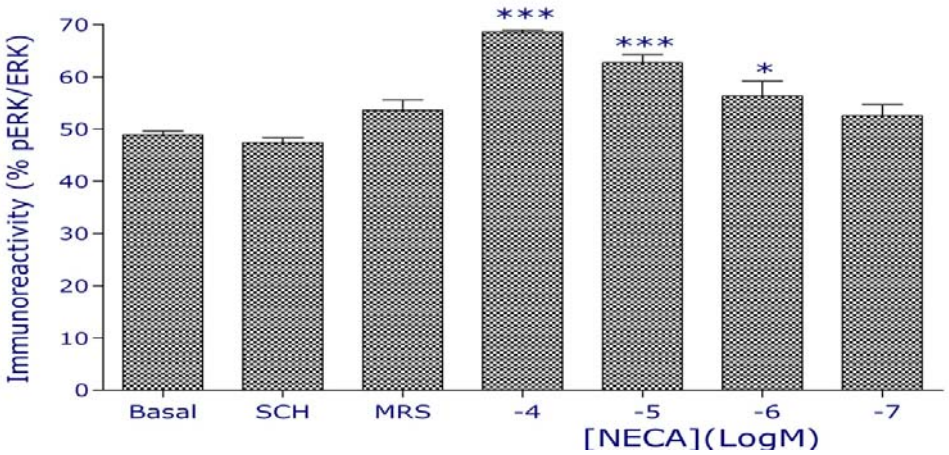

Figure 6 Quantification of phosphoMAPK bands, from immunoblots of SH-SYSY cells stimulated with increasing concentrations of NECA (100, 10, 1 µM and 100 nM) for 5 min in the presence of 1 µM SCH58261, or antagonists alone (SCH58261 & MRS1754) added to a final concentration of 1 µM. Results are expressed as a percentage of total ERK1/2; data are means ± SEM of three independent experiments, expressed as a percentage of total ERK1/2. *P<0.05 & ***P<0.001 compared to basal. Data were analysed for statistical significance using one way ANOVA with Bonferonni's Multiple Comparison test.

DISCUSSION

This study has assessed functional expression of A_{2B} adenosine receptors in the human neuroblastoma SH-SY5Y cell line. The findings in this study suggest the expression A_{2B} adenosine receptors in SH-SY5Y cells, coupling to mitogensis signalling pathways. An early report prior to general acceptance of the division of A_{2A} and A_{2B}, described an increased cAMP formation in response to ADO agonists in the human neuroblastoma IMR32 cell line, with the consequent suggestion that they expressed A_2, with no subtype distinction (Abbracchio et al.1989). The human neuroblastoma NG108-15 has been suggested to co-express A_{2A} and A_{2B}adenosine receptor, with an increase in AC activity upon NECA stimulation (Mundell, Kelly 1998, Mundell 1998).

A_{2B} have long been characterised as coupling primarily to G_s protein and the elevation of cAMP. Recent studies indicate that A_{2B} can also couple to G_q to produce Ca^{2+} mobilization and MAPK activation (Linden et al.1999). Ca^{2+} mobilization is not limited to cells that over-express A_{2B}, because endogenous A_{2B} in HEK-293 cells are also able to produce a robust A_{2B}-mediated Ca^{2+} mobilization (Teng et al.2005, Gao et al.1999).This alternative signalling pathway for A_{2B} coupling to $[Ca^{2+}]_i$ levels was investigated in SH-SY5Y cells. However, calcium measurements revealed a lack of response to NECA, suggesting that A_{2B} is not coupled via G_q protein in SH-SY5Y cells. The endogenous muscarinic receptor, however, showed a marked calcium response in the presence of 1 µM carbachol (Figures 1 and 2).

In HEK-293 cells, NECA was reported to be equipotent in elevating cAMP and stimulating ERK1/2 MAPK activation. The PKA inhibitor, H89, blocked forskolin, but not NECA, activation of MAPK in HEK cells, with the suggestion that A_{2B} receptors couple via the G_q pathway to MAPK activation through a pathway including MEK and Ras (Gao et al.1999). Another report indicated increases in ERK1/2 phosphorylation at NECA concentrations in the nanomolar range in A_{2B} transfected CHO cells, which were abolished by CGS15943 (Schulte, Fredholm 2000). Another alternative pathway for A_{2B} signalling is, therefore, the MAPK pathways. Administration of single concentrations of (1 µM) AR antagonists (A_{2A} selective antagonist: SCH58261, non-selective AR antagonist with A_{2A} high affinity: CGS15943 and A_{2B} selective antagonists: MRS1754) in the presence of 10 µM NECA or CGS21680 confirmed that the MAPK responses to adenosine agonists stimulation are receptor mediated (Figure 3). A_{2B} appear to couple to stimulation of ERK1/2 MAP kinases in SH-SY5Y cells in concentration- and time-dependent manner (Figure 4 & 6), in that NECA evoked an increase in ERK1/2 phosphorylation with no evidence for stimulation of p38 or JNK MAP kinases (Figure 4). These responses were also conducted in the presence of SCH58261(Arslan, Fredholm,2000) to prevent any potential A_{2A} modulation of MAP kinase activity (Figure 5). Data from immunoblotting suggest a better characterisation of the anti-p38 antibody is required, exploiting positive controls. An alternative explanation is that the phospho-p38 may be expressed at too low a density for successful immunoblotting.

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

Because A_{2B} are relatively insensitive to ADO, they may become activated, or remain activated, only when other AR have been completely activated (and potentially desensitized) by high levels of ADO, as might be apparent in pathophysiological conditions. Thus it might be the case that higher concentrations may lead to an emphasis on the A_{2B}-mediated ERK1/2 activation (potentially mitogenic) to alter the pattern of gene expression and cell proliferation and differentiation (Haas and Selbach 2000). In summary, our results demonstrate that the expression of adenosine receptors by the human neuroblastoma cell line SH-SY5Y, coupled to distinct signalling mechanisms, with the pharmacological profiles of A_{2B} subtypes.

REFERENCES

Abbracchio MP, Cattabeni F, Clementi F, Sher E.(1989). Adenosine receptors linked to adenylate cyclase activity in human neuroblastoma cells: modulation during cell differentiation. Neuroscience;30(3):819-25.

Alexander SP, Mathie A, Peters JA. (2008). Guide to Receptors and Channels (GRAC), 3rd edition. Br J Pharmacol Mar;153 Suppl 2:S1-209.

Arslan G, Fredholm BB. (2000) Stimulatory and inhibitory effects of adenosine A(2A) receptors on nerve growth factor-induced phosphorylation of extracellular regulated kinases 1/2 in PC12 cells. Neurosci Lett Oct 13;292(3):183-6.

Bruns RF, Lu GH, Pugsley TA. (1986). Characterization of the A2 adenosine receptor labeled by [3H]NECA in rat striatal membranes. Mol Pharmacol Apr;29(4):331-46.

Dickenson JM, Blank JL, Hill SJ. (1998). Human adenosine A1 receptor and P2Y2-purinoceptor-mediated activation of the mitogen-activated protein kinase cascade in transfected CHO cells. Br J Pharmacol Aug;124(7):1491-9.

Feoktistov I, Goldstein AE, Biaggioni I. (1999). Role of p38 mitogen-activated protein kinase and extracellular signal-regulated protein kinase kinase in adenosine A2B receptor-mediated interleukin-8 production in human mast cells. Mol Pharmacol Apr;55(4):726-34.

Feoktistov I, Goldstein AE, Biaggioni I. (1999). Role of p38 mitogen-activated protein kinase and extracellular signal-regulated protein kinase kinase in adenosine A2B receptor-mediated interleukin-8 production in human mast cells. Mol Pharmacol Apr;55(4):726-34.

Feoktistov I, Murray JJ, Biaggioni I.(1994). Positive modulation of intracellular Ca2+ levels by adenosine A2b receptors, prostacyclin, and prostaglandin E1 via a cholera toxin-sensitive mechanism in human erythroleukemia cells. Mol Pharmacol Jun;45(6):1160-7.

Fredholm BB, Arslan G, Halldner L, Kull B, Schulte G, Wasserman W. (2000). Structure and function of adenosine receptors and their genes. Naunyn Schmiedebergs Arch Pharmacol Nov;362(4-5):364-74.

Gao Z, Chen T, Weber MJ, Linden J. (1999). A2B adenosine and P2Y2 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 Feb 26;274(9):5972-80.

Graham S, Combes P, Crumiere M, Klotz KN, Dickenson JM. (2001). Regulation of p42/p44 mitogen-activated protein kinase by the human adenosine A3 receptor in transfected CHO cells. Eur J Pharmacol May 18;420(1):19-26.

Haas HL, Selbach O. (2000). Functions of neuronal adenosine receptors. Naunyn Schmiedebergs Arch Pharmacol Nov;362(4-5):375-81.

Hancock JT. (2005). Cell Signalling. second edition ed. Oxford University Press, Oxford, Uk.;

Hide I, Padgett WL, Jacobson KA, Daly JW. (1992). A2A adenosine receptors from rat striatum and rat pheochromocytoma PC12 cells: characterization with radioligand binding and by activation of adenylate cyclase. Mol Pharmacol Feb;41(2):352-9.

Hunter T.(1995). Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling. Cell Jan 27;80(2):225-36.

Jackson AM, SJ, J. HSJ, Alexander SPH. (2003). Investigation of endogenous A2B adenosine receptor-evoked Ca2+ signalling in human embryonic kidney (HEK) cells using a fluorescence integrated plate reader (FLIPR). Br J Pharmacol ;pA2 online.:1, 72P.

Linden J, Thai T, Figler H, Jin X, Robeva AS. (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 Oct;56(4):705-13.

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

Miloso M, Villa D, Crimi M, Galbiati S, Donzelli E, Nicolini G, et al. (2004). Retinoic acid-induced neuritogenesis of human neuroblastoma SH-SY5Y cells is ERK independent and PKC dependent. J Neurosci Res Jan 15;75(2):241-52.

Mirabet M, Herrera C, Cordero OJ, Mallol J, Lluis C, Franco R. (1999). Expression of A2B adenosine receptors in human lymphocytes: their role in T cell activation. J Cell Sci Feb;112 (Pt 4):491-502.

Mundell SJ, Kelly E. (1998). Evidence for co-expression and desensitization of A2a and A2b adenosine receptors in NG108-15 cells. Biochem Pharmacol Mar 1;55(5):595-603.

Mundell SJ, Luty JS, Willets J, Benovic JL, Kelly E. (1998). Enhanced expression of G protein-coupled receptor kinase 2 selectively increases the sensitivity of A2A adenosine receptors to agonist-induced desensitization. Br J Pharmacol Sep;125 (2):347-56.

Olah ME, Stiles GL. (1995). Adenosine receptor subtypes: characterization and therapeutic regulation. Annu Rev Pharmacol Toxicol ;35:581-606.

Olah ME.(1997). Identification of A2a adenosine receptor domains involved in selective coupling to Gs. Analysis of chimeric A1/A2a adenosine receptors. J Biol Chem Jan 3;272(1):337-44.

Ralevic V, Burnstock G. (1998). Receptors for purines and pyrimidines. Pharmacol Rev Sep;50(3):413-92.

Robinson AJ, Dickenson JM. (2001). Regulation of p42/p44 MAPK and p38 MAPK by the adenosine A(1) receptor in DDT(1)MF-2 cells. Eur J Pharmacol Feb 16;413(2-3):151-61.

Schulte G, Fredholm BB. (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 Sep;58(3):477-82.

Seidel MG, 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 Sep 3;274(36):25833-41.

Sexl 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 Feb 28;272(9):5792-9.

Teng B, Qin W, Ansari HR, Mustafa SJ.(2005). Involvement of p38-mitogen-activated protein kinase in adenosine receptor-mediated relaxation of coronary artery. Am J Physiol Heart Circ Physiol Jun;288(6):H2574-H2580.

Yoshimura Y, Kristo G, Keith BJ, Jahania SA, Mentzer RM, Jr., Lasley RD. (2004). The p38 MAPK inhibitor SB203580 blocks adenosine A(1) receptor-induced attenuation of in vivo myocardial stunning. Cardiovasc Drugs Ther Nov;18(6):433-40.