CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1252397

Available online at: http://www.iajps.com Research Article

SELECTIVE AND NON-SELECTIVE ACTIVATED AND INHIBITORY AGENTS EFFECTS ON ADENYLYL CYCLASE IN THE KIDNEY OF THE RATS

[1]Muhammad Kamran, [2]Dr. Awais Butt, [3]Shahzaib Nawaz
Medical officer at Trauma Centre Phool Nagar, Kasur, Pakistan
Medical Officer THQ Hospital Sangla Hill
Jinnah Hospital Lahore

Abstract:
Objective: *To have in depth knowledge about the effects of nonselective and selective inhibitory and activated agents on adenylyl cyclase in rat kidney.*
Design: *A variety of concentrations of pharmacological agents were prepared. They include nebularine, Ap3A, forskolin, Ap4A and caffeine. Furthermore, effects of these agents were noted in relation to rat kidney adenylyl activity.*
Methods: *Tissue of rat kidney was used in the process of preparation of crude extract. Activity of adenylyl cyclase in connection with crude extract was observed. [2-H3] ATP was used as substrate which ultimately lead to the formation of cAMP. Pharmacological agents with their prepared concentrations were tested. Prominent activator of adenylyl cyclase, forskolin, was selected as a compound. Ap4A, caffeine, nebularine, and Ap3A were utilized for comparison purpose.*
Results: *Adenylyl cyclase activity was at peak at 100 M forskolin as per concluded results. Nebularine inhibited activity of enzyme when agent concentration enhanced up to 50 M where the inhibition started to stable. No considerable effect on the enzyme activity in kidney tissue was observed when caffeine with 10 – 300 M on the of adenylyl cyclase activity was used. No effect on adenylyl cyclase activity was noted when Ap3A over the concentration range of 10 – 300 M was used. However, an inhibition effect on the enzyme activity was noted when Ap4A with the concentration 100 M was used.*
Conclusion: *Role of cyclic nucleotides in metabolism control and cell-signaling is undeniably significant. It stimulates inhibitors and activities of cyclase to make some likely physiological impacts. Foreskin being initiator of adenylyl cyclase, Ap4A and nebularine are established to be latent inhibitors of cyclase, which signifies their importance in curing schizophrenia, mania, seizure etc.*
Key Words: *Ap3A, Nucleotides, Caffeine, Forskolin, Ap4A, Nebularine. Adenylyl cyclase*

Corresponding author:
Muhammad Kamran,
Medical officer at Trauma Centre Phool Nagar,
Kasur, Pakistan

QR code

Please cite this article in press Muhammad Kamran et al., ***Selective and Non-Selective Activated and Inhibitory Agents Effects on Adenylyl Cyclase in the Kidney of the Rats,*** *Indo Am. J. P. Sci, 2018; 05(05).*

INTRODUCTION:

Cyclic nucleotides have a significant role in metabolic control and cell-signaling. Inhibitors and activators related to cyclase are those agents which have their worth in pharmacology. We are already quite aware of these compounds. Forskolin is a case in point in this regard. Activation created by forskolin is investigated in the study at hand. Other potential inhibitors and activators in connection with their effects on kidney adenylyl cyclase were also tested. The obtained results in terms of the effects of these compounds i.e. Ap3A, nebularine, Ap4A and caffeine, were compared to those of forskolin. By excluding forskolin, all compounds under investigation were purine derivatives. Nebularine is a purine nucleoside antibiotic. Purine 9-D-ribofuranoside is a structure of Nebularine. This particular inhibitor is entirely rare in naturally available purine ribosides. Production of compound as a normal metabolite is done by fungus Lepista (Clitocybe) Nebularis. It possesses natural potency just like cytotoxin. Inhibition of saplings growth of diverse species is carried out by it. It is also responsible for mitotic abnormality in tips of the roots. Influenza B virus multiplication is inhibited too by it [1]. Being enormously poisonous for guinea-pigs [2] and mice, nebularine creates mitotic aberrations i.e. breakdown of chromosome. Toxicity in animal cells is inhibited when ATP, AMP, and adenosine with concentrations 100-fold that of nebularine are used. Inhibition of adenosine deaminase and NAD+ - reliant glucose dehydrogenase is also carried out by Nebularine [3]. Caffeine is derived mainly from coffee and tea. Being methylated xanthine, it is observed to block phosphodiesterase's [4]. Ap4A and Ap3A are considered as alarm ones because they carry out regulation of metabolism of cells, thus indicating the signals to cells to adjust in new environment. There is a proof that Ap4A works just like a positive regulator for DNA duplication [6]. Human platelets contain massive amount of Ap4A and Ap3A. When platelets are activated, these two dinucleotides are freed to join superfluous cellular milieu, where their role in the regulation of the Vanstone and modulation of platelet aggregation is significant [7].

MATERIAL AND METHODS:

Sigma Chemical Co. was utilized to get cyclic nucleotides, biochemicals and column chromatography materials. Amersham International plc was used to obtain [23H] adenosine-5-triphosphate i.e. Ammonium salt. Repeatedly, the same source (Amersham International plc) was utilized to get high efficiency 'Ready Safe' scintillation cocktail having phenylxylylethane surfactant. Wister rats (male) utilized to obtain mammalian tissue, weighing from 250 to 350grams in average. These were chosen randomly from a rat colony from a well-known international hospital. The obtained rats were placed further in animal unit a well-known international hospital. Air conditioned (24 degree centigrade) room was provided to them. 12 hours dark/light cycle was maintained too. Arrangement of feeding consisting of standard food, were carried out from Grain, Silos and Flour Mills.

Extraction of adenylyl cyclase activity

Collected sampling of tissues were kept in cold place i.e. ice and were sliced into fragments as per requirements. Homogenization was completed in three minutes in ice-cold, 45mM Tris-HCl (containing pH 07.40) consisting of 8 mM theophylline, 0.25M sucrose and 6mM 2-mercapto-ethanol at highest speed i.e. fifteen hundred revolutions per minute. Nine milliliters of buffer were utilized for every g of tissue. Centrifugation of homogentaion was performed at 3000gram for 10mintues at 2 Centigrade. The precipitate was disposed of while Re-centrifugation of supernatant was done at 150,000 grams for 40mintues at temperature two degree centigrade. The concluding precipitate was re-suspended in two to three milliliters of 45mM consisting of 8 mM theophylline and 6mM 2-mercaptoethanol. Division of it was done into two parts. Out of two parts, one was used to find out protein while other was utilized for examination of adenylyl cyclase activity [9]. After slight adjustments, procedure of Alvarez and Daniels was utilized [10]. In incubations of assay, 20µl of the probable inhibitors or activators solution of dissimilar concentrations replaced 20µl of the Tris-HCl buffer.

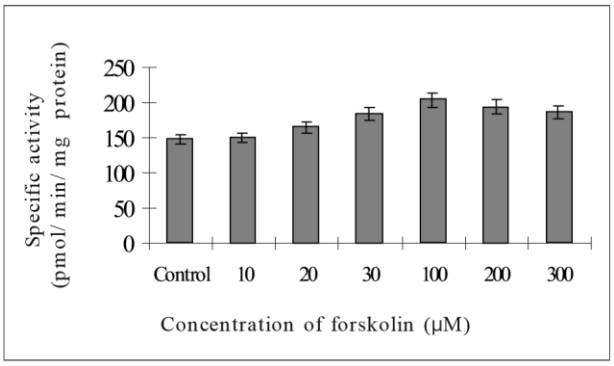
RESULTS:

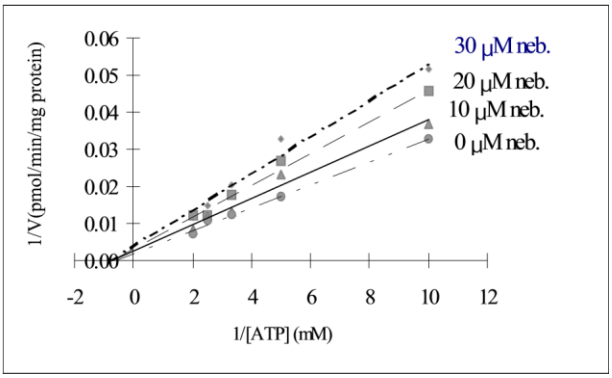
Adenylyl cyclase activity was at peak at 100 M forskolin as per concluded results. Nebularine inhibited activity of enzyme when agent concentration enhanced up to 50 M where the inhibition started to stable. No considerable effect on the enzyme activity in kidney tissue was observed when caffeine with 10 – 300 M on the of adenylyl cyclase activity was used. No effect on adenylyl cyclase activity was noted when Ap3A over the concentration range of 10 – 300 M was used. However, an inhibition effect on the enzyme activity was noted when Ap4A with the concentration 100 M was used.

DISCUSSION:

Present study confirmed that peak activity of adenylyl cyclase was at 100 M forskolin as in (Figure. 1). This corresponds to the already known

reports on kidney tissue [11, 12]. In Figure.2, inhibition of adenylyl cyclase activity by nebularine is demonstrated. In the earlier stage when nebularine concentration was enhanced, a quick inhibition activity was noted up to 50µM nebularine. The inhibition was noted as 66 percent. Inhibition was stable from 50 – 300 µM nebularine. Kinetic study demonstrated that inhibition was non-competitive when inhibitor concentration was ranging from 0 ,10, 50, 300µM and substrate concentrations ranged from 0.1 to 0.5mM (Figure. 3). Nebularine result on adenylyl cyclase is akin to the result on xanthine oxidase i.e. main enzyme of purine catabolism) [13].





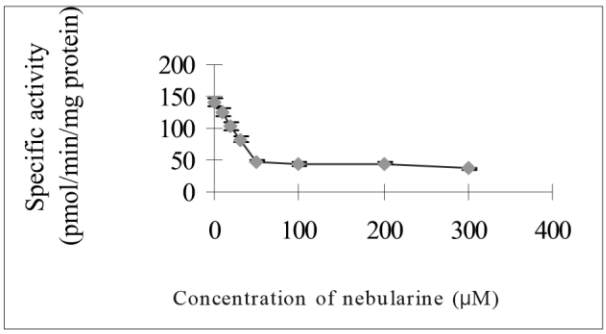


Figure-2: The inhibitory effect of nebularine on the adenylyl cyclase activity (pmol/ min/ mg protein) of rat kidney. 0.5mM [2-H^3] ATP was used as substrate. Incu-

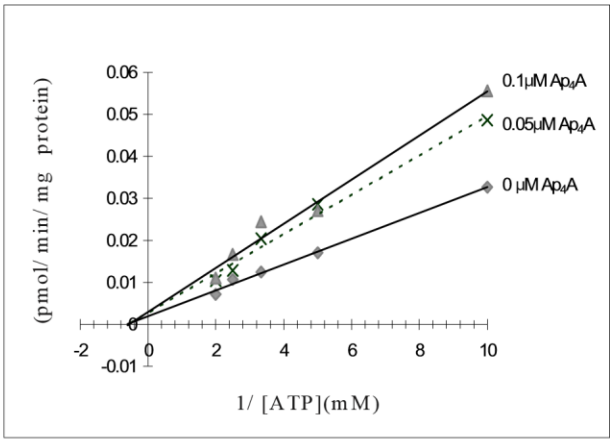


Figure-4: Lineweaver-Burk plot of adenylyl cyclase kinetics in the presence of Ap_4A. The enzyme was in 45mM

Figure – 1 & 2: The stimulatory effect of forskolin on the adenylyl cyclase activity (pmol/ min/ mg protein) of rat kidney. 0.5mM [2-H^3] ATP was used as substrate. Incubation contained 4mM $MgSO_4$, 45 mM Tris-HCl buffer at pH 7.4. Data points are each the mean of 12 replicate determination (±SD).

Figure – 3 & 4: Lineweaver-Burk plot of adenylyl cyclase kinetics in the presence of nebularine. The enzyme was in 45 mM Tris- HCl buffer (pH 7.4) containing 10 mM theophylline. Data points are each of the mean of 12 replicate determination.

This outcome of inhibition of nebularine in relation to activity of adenylyl cyclase can be because of GTP- binding regulatory protein effect, e.g. dissociation of the alpha subunit of the stimulatory GTP- binding regulatory protein can be prevented by nebularine. In in vivo, nebularine may block hormone stimulating adenylyl cyclase by stopping receptor. Inhibitory effects of nebularine in relation to adenylyl cyclase prove its vitality as a compound in pharmacological and laboratory procedures. Thus, possibility of the compound in treating schizophrenia, mania and seizure etc. cannot be doubted. By carrying an effective potency of inhibition in case of adenylyl cyclase, this compound can be taken as selective inhibitor. No considerable effect on the enzyme activity in kidney tissue was observed when caffeine with 10 – 300 M on the of adenylyl cyclase activity was used(Table-I). However, acquired result seems in comparison to Sheppard reports [14] and Jakobs et al. [15]. It is pertinent to note that caffeine is found to be effective in inhibiting the cAMP-

induced activation of adenylyl cyclase when it stops signal transduction. It does not act directly on enzymes [16]. Kubota and Oyama [17] observed in the saponin-treated cells, caffeine subdued the cation-induced activation of adenylyl cyclase. After chronic caffeine ingestion in mice, forskolin, nonetheless, did not affect stimulation of striatal adenylyl cyclase [18].

TABLE-I: Caffeine effect on activity of adenylyl cyclase i.e. pmol/min/mg protein, from kidney of rat. 0.5 mM (2 – H3) ATP and 4mM MgSO4 were combined in incubation mixture. 45 mM Tris-HCl buffer which contained pH 7.4, was used to extract kidney tissue without having 10mM theophylline. Collected data was the mean of five rats. Every determination was in triplicate (±SD).

Caffeine (µM) final concentration	Specific activity (pmol / min / mg protein)	Change relative to control (%)
0 (Control)	114 ± 21	0
10	109 ± 18	-5
20	113 ± 19	-1
30	113 ± 15	-1
100	112 ± 16	-2
200	110 ± 13	-4
300	114 ± 17	0

TABLE-II: Ap3A effect on the activity of adenylyl cyclase i.e. pmol/min/mg protein, from kidney of rat. 0.5 mM (2-H3) ATP and 4 mM MgSO4 were combined in incubation mixture. 45 mM Tris-HCl buffer which contained pH 7.4, was used to extract kidney tissue having 10 mM theophylline. Collected data was the mean of five rats. Every determination was in triplicate (±SD).

Ap_3A (µM) final concentration	Specific activity (pmol / min / mg protein)	Change relative to control (%)
0 (Control)	112 ± 15	0
10	112 ± 11	0
20	113 ± 14	-1
30	103 ± 12	-8
100	104 ± 13	-7
200	107 ± 6	-5
300	107 ± 8	-5

Table – III: Ap4A inhibitory effect on adenylyl cyclase activity i.e. pmol/min/mg protein, from kidney of rat. 0.5 mM (2 – H3) ATP and 4mM MgSO4 were combined in incubation mixture. Kidney buffer, having pH 7.4, contained 10 mM theophylline. Collected data was the mean of five rats. Every determination was in triplicate (±SD).

Ap_4A (µM) final concentration	Specific activity (pmol / min / mg protein)	Change relative to control (%)
0 (Control)	125 ± 15	0
10	117 ± 7	- 6
20	115 ± 13	-8
30	104 ± 6	-17
50	98 ± 4	-22
100	81 ± 5	-35
200	146 ± 17	17
300	133 ± 18	6
1000	123 ± 12	-2

The mixtures of (Ap4A and Ap3A) and (adenylyl cyclase incubation) were obtained one by one. No effect on adenylyl cyclase activity was noted when Ap3A over the concentration range of 10 – 300 M was used. However, an inhibition effect on the enzyme activity was noted when Ap4A with the concentration 100 M was used. These results indicate that Ap4A and nebularine are latent inhibitors of cyclase, which signifies their importance in curing schizophrenia, mania, and seizure.

CONCLUSION:

Role of cyclic nucleotides in metabolism control and cell-signaling is undeniably significant. It stimulates inhibitors and activities of cyclase to make some likely physiological impacts. Foreskin being initiator of adenylyl cyclase, Ap4A and nebularine are established to be latent inhibitors of cyclase, which signifies their importance in curing schizophrenia, mania, seizure etc.

REFERENCES:

1. Tamm I, Folkers K & Shunk CH. A certain benzimidazoles, benzenes, and ribofuranosylpurines as inhibitors of influenza B virus multiplication, J Bacteriol. 1956;72: 59-62.
2. Truant A P &D' amato H E. Pharmacologic and toxicologic actions of nebularine, Fed Proc. 1955; 14:391-5.
3. Nair V & Weichert RJ. Substrate specificity of adenosine deaminase-function of the 5´-hydroxylgroup of adenosine, Bioorg. Chem. 1980; 9: 423-7.
4. Drummond GI & Duncan L. Adenyl cyclase in cardiac tissue, J. Biol. Chem. 1970; 245: 976-83.
5. Bochner BR., Lee PC, Wilson SW, Cutler CW &Ames BN. Apppp A and related adenylated nucleotide are synthesized aa consequence of oxidation stress, Cell 1984;37: 225-32.
6. Zamecnik PC. Diadenosine 5',5'"- P1, P4-tetraphosphate (Ap4A): its role in cellular metabolism, Anal. Biochem. 1983; 134: 1-10.
7. Lüthje J. & Ogilvie A. Catabolism of Ap4A and Ap3Ain whole blood, Eur J Biochem. 1988; 173: 241-5.
8. Kimura T, Hatano N, Wada M, Iwata K, Kurosaki Y, Nakayama T, Yamaura T & Nakajima H. Disposition of diadenosine 5',5'"- P1, P4-tetraphosphate (Ap4A) in rats, Biol Pharm Bull. 1995; 18(11): 1556-9.
9. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal Chem. 1976; 72: 248-54.
10. Alvarez R & Daniels DV. A separation method for the assay of adenylyl cyclase, intracellular cyclic AMP, and cyclic-AMP phosphodiesterase using tritium-labeled substrates, Anal Biochem. 1992; 203: 76-82.
11. Takats A, Binh VH & Bertok L. Potential role of SH group in the radio senssitivity of adenylate cyclase, Acta Physiol Hung. 1990; 76(4): 265-72.
12. Marano I, Adler K, Weismann K, Knorr A, Erdmann E& Bohm M. Correlation of myosin heavy chain expression in the rat with cAMP in different models of hypertension-induced cardiac hypertrophy, J Mol Cell Cardiol. 1993; 25(4): 387-94.
13. Brown E & Konuk M. Plant cytotoxicity of nebularine (purine riboside), Phytochemistry. 1994; 37(6):1589-92.
14. Sheppard H. Inhibition of norepinephrine stimulated adenyl cyclase by theophylline, Nature (London) 1970;228: 567-71.
15. Jakobs KH, Schultz K & Schultz G. Inhibition of adenyl cyclase preparations from rat kidney by calcium ions and various diuretic, Nauryn. Schmiedeberg's Arch Pharmacol. 1972; 273: 248-55.
16. Brenner M & Thoms SD. Caffeine blocks activation of cyclic AMP synthesis in Dictyosteliumdiscoideum, Dev Biol. 1984; 101:136-46.
17. Oyama M & Kubota K. Activation of adenylate cyclase by divalent cations and polyamines in saponin treated Dictyosteliumdiscoideum cells, J Biochem. Tokyo, 1995; 118(1): 117-21.
18. Shi D, Nikodijevic O, Jacobson KA & Daly JW. Effects of chronic caffeine on adenosine, dopamine and acetylcholine systems in mice, Arch Int Pharmacodyn Ther. 1994; 328(3): 261-87.