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REVIEW

Adenosine, Oxidative Stress and Cytoprotection

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ABSTRACT—Adenosine, a metabolite of ATP, serves a number of important physiological roles in the body. These actions contribute to sedation, bradycardia, vasorelaxation, inhibition of lipolysis and regulation of the immune system and are mediated, in part, through activation of three distinct adenosine receptor (AR) subtypes. To date, four receptor types have been cloned: A_1, A_{2A}, A_{2B} and A_3. It is becoming increasing clear that adenosine contributes significantly to cytoprotection, a function mediated principally by the A_1AR and A_3AR. In this review, we survey the literature on the role of adenosine and the mechanisms underlying cytoprotection and ischemic preconditioning, a process characterized by cytoprotection derived from repeated brief ischemic challenges. An important recent observation is that the expression of several AR subtypes could be regulated by oxidative stress to provide a greater cytoprotective role. Thus, like other proteins known to be regulated during ischemia, the A_1AR and A_3AR can be considered as being inducible receptors.

Keywords: Adenosine, Cytoprotection, Oxidative stress, Adenosine receptor, Nuclear factor kappa B

1. Introduction

Adenosine and ATP are important components of the purinergic system, mediating their effects preferentially at the P_1 and P_2 purinoceptors, respectively. While not considered a true neurotransmitter, since it is not stored in synaptic vesicles, adenosine serves important autocrine and paracrine roles. One such role, the regulation of cardiovascular functions, was demonstrated over 70 years ago by Drury and Szent-Gyorgyi (1). These investigators demonstrated that adenyl purines were important for promoting coronary vasodilation, suppressing heart rate and decreasing blood pressure. Subsequent studies by Berne (2) demonstrated a vasodilatory role of adenosine elaborated during hypoxia. In addition to its cardiovascular role, adenosine also suppresses central nervous system excitability, inhibits lipolysis, provokes bronchoconstriction, suppresses the generation of superoxides in neutrophils and decreases glomerular filtration rate. However, one of the primary roles of this nucleoside is cytoprotection. Adenosine, released under conditions of stress, provides negative feedback regulation to maintain cellular preservation and is therefore termed a "retaliatory metabolite."

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2. Adenosine and adenosine receptors

Synthesis of adenosine

The levels of adenosine are determined primarily from the dephosphorylation of its immediate precursor, adenosine monophosphate (AMP). Precursors of AMP include cyclic AMP, ADP and ATP. ATP is co-released with other neurotransmitters from presynaptic vesicles and is also produced by mast cells, basophils and endothelial cells and as a result of cellular damage. ADP is derived from activated platelets, while cyclic AMP serves as a second messenger in most cells. Since the estimated ratio of ATP:AMP under normoxic condition is approximately 50:1, a small decrease in total ATP is expected to produce a large increase in AMP and adenosine (3). Adenine nucleotides are degraded by a series of ectonucleotidases. One such enzyme, 5'-nucleotidase, catalyzes the conversion of AMP to adenosine during increased cellular metabolism. 5'-Nucleotidase is found both extracellularly (attached to the plasma membrane by glycosyl-phosphatidylinositol anchors) and in the cytosol (3, 4). Regulation of the activity and/or expression of this enzyme is critical for regulation of the levels of adenosine. The initial report suggested that the level of ecto-5'-nucleotidase and adenosine were increased by ischemic preconditioning (5). However, subsequent studies using microdialysis were unable to confirm this result (6, 7). Adenosine is also produced from the hydrolysis of

S-adenosylhomocysteine, by *S*-adenosylhomocysteine hydrolase. This mechanism accounts for a significant portion of the adenosine present under resting conditions. *S*-Adenosylhomocysteine also serves as an intracellular binding protein for adenosine, thereby protecting the nucleoside from degradation. In the central nervous system, *S*-adenosylhomocysteine is localized on glial and astrocytic cells near to synaptic terminals. Adenosine is rapidly cleared from the extracellular space through a bi-directional facilitated transporter that is sensitive to the drug dipyridamole. As such, application of dipyridamole increases interstitial adenosine levels, thereby accounting for the pharmacological actions of this drug, which include coronary vasodilation, sedation and anticonvulsant action. Under normoxic conditions, adenosine is phosphorylated by adenosine kinase to AMP and subsequently to ATP to restore the nucleotide pool. However, under conditions of increased metabolic stress, the increased levels of adenosine easily saturate adenosine kinase and excess adenosine is metabolized to inosine and hypoxanthine by adenosine deaminase (3, 4).

Adenosine receptors

Adenosine exerts its major physiological roles by interacting with various subtypes of P_1 purinergic receptors, also termed adenosine receptors (ARs). These receptors differ from the P_2 purinergic receptors based on their preference for adenosine over ATP (8). The AR subtypes that have currently been described from both functional and molecular cloning studies include the A_1, the A_{2A}, the A_{2B}, and the A_3 subtypes. The A_1AR represents the predominant AR subtype in the central nervous system (3). It differs from other AR subtypes based on a unique agonist interaction profile: the order of potency for agonists with decreasing affinity for the A_1AR is *R*-N^6-phenylisopropyladenosine (*R*-PIA) > 5'-*N*-ethylcarboxamidoadenosine (NECA) > *S*-PIA (9). Methylxanthines, such as theophylline, caffeine and 3-isobutyl-1-methylxanthine, are generally nonselective antagonists of most ARs subtypes. However, the rat A_3AR appears relatively insensitive to methylxanthines (10). A number of substituted 8-phenylxanthine derivatives, such as 8-(4-[([[(2-aminoethyl)amino] carbonyl]methyl)oxy]phenyl)-1,8-diallylxanthine (XAC), are selective antagonists for the A_1AR.

Autoradiographical studies indicate wide distribution of the A_1AR, particularly to the cortex, cerebellum (both molecular and granular layers), the hippocampus and thalamus (3). Immunohistochemical studies have shown high levels of A_1AR in the central auditory system, including the temporal cortex, medial geniculate, inferior colliculus and cochlear nucleus (11). In the periphery, high levels of the A_1AR are detected in the testis and adipose tissue, with lower levels in the kidney and heart. Activation of the A_1AR mediates inhibition of adenylyl cyclase in a number of systems through activation of the G_i proteins. Using purified preparations derived from bovine brain, preferential coupling of the A_1AR to the G_{i3} protein was demonstrated, with lower affinities for G_{i2}, and G_o proteins (12). The A_1AR inhibits presynaptic voltage-sensitive Ca^{2+} channels in cortical synaptosomes (13), hippocampal neurons (14) and Ca^{2+} currents into rat dorsal root ganglion cells (15). Such inhibition of Ca^{2+} influx could subserve, in part, the cytoprotective role of adenosine. Adenosine also activates 4-aminopyridine-sensitive K^+ channels in hippocampal neurons and a K^+ conductance in atrial myocytes (16).

The A_1AR was purified, prior to its cloning, from rat testes (17) and bovine brain (18). In most tissues, this receptor exhibits a molecular size of 36 kDa, while in the testis it migrates as a 42-kDa protein (19). This larger apparent molecular size is a result of increased glycosylation of the A_1AR in the testis (17).

3. Adenosine mediates cytoprotection

Adenosine serves a paracrine role since it is released in response to ischemic stress and activates cells in the vicinity of its release site. During low-flow ischemia, the levels of cardiac interstitial adenosine increases from submicromolar levels to micromolar concentrations (20). The nucleoside mediates its cytoprotective action by interacting with A_1AR and A_3AR on myocardial tissue and central nervous system. In the central nervous system, experiments utilizing microdialysis technique indicate a good correlation between the levels of extracellular adenosine and cerebral blood flow. For example, a transient increase in adenosine was observed when flow was reduced below 25 ml · 100 g^{-1} · min^{-1} (21).

Cardiovascular system

In the cardiovascular system, adenosine plays an important role in reducing cellular injury produced during ischemia and reperfusion. Several mechanisms of cytoprotection have been proposed. The nucleoside reduces heart rate and force of contractility (22, 23), thereby reducing the oxygen demand of the heart. Adenosine also reduces the local build-up of reactive oxygen species (ROS) through inhibition of superoxide anion generation by neutrophils (24) and increases myocardial glucose uptake (25, 26), probably secondary to the increase in coronary blood flow (27). Furthermore, adenosine serves as a substrate for the regeneration of ATP, mediated via the purine salvage pathway described above. The direct action of adenosine to suppress heart rate and contractility can be explained by the ability of this nucleoside (via the A_1AR) to activate the acetylcholine-sensitive K^+ channels in the atrium (16) and to inhibit catecholamine-stimulated cyclic AMP generation (28) and Ca^{2+} channels in the ventricle (29).

Central nervous system

Another area in which the cytoprotective action of adenosine has been studied extensively is the central nervous system. The levels of adenosine in the central nervous system increase significantly following metabolic insults such as ischemia, hypoxia and hypercapnea (30, 31). While these increases confer some degree of neuroprotection, exogenously applied adenosine analogs can provide additional protection against these injuries. For example, administration of 2-chloroadenosine to rats protected against hippocampal cell loss induced by ischemia (32). Another analog, cyclohexyladenosine, protected against cerebral ischemia in gerbils (33) and against transient ischemia in rats (34). Cyclohexyladenosine also provided protection to the hippocampus and striatum following 30 min bilateral carotid occlusion (34). Rats treated with caffeine to increase A_1AR expression in the brain were more resistant to ischemia, underscoring the protective role of this receptor subtype (35). In contrast, down-regulation of the A_1AR by prolonged agonist treatment exacerbated the damage created by a subsequent ischemic episode (36). While the central nervous system protective role of adenosine has been attributed to the A_1AR, an additional role of the A_3AR has not been ruled out (36). In contrast to the A_1 and A_3AR, activation of the A_{2A}AR in the brain appears to aggravate ischemia (37).

Proposed mechanisms underlying adenosine-mediated cytoprotection

Several mechanisms contributing to the cytoprotective role of adenosine in the central nervous system have been proposed. The major mechanism proposed involves activation of presynaptic A_1AR to decrease release of excitatory neurotransmitter such as glutamate (38 – 40). These presynaptic A_1AR activate a K^+ conductance (leading to hyperpolarization) (41, 42) and inhibit Ca^{2+} influx into the nerve terminal (11, 34). Such actions reduce neuronal excitability and firing rate (43). Activation of a voltage-dependent Cl^- conductance by adenosine, distinct from that activated by GABA, can also contribute to neuronal hyperpolarization (44). Adenosine also acts postsynaptically to reduce NMDA receptor-induced synaptic amplification (45) and hyperpolarizes astrocytes (46), thereby facilitating glutamate uptake by these cells. Furthermore, adenosine analogs decrease the rate of glucose consumption in the central nervous system (47, 48), due in part to inhibition of neuronal activity (49) and through augmentation of cerebral blood flow through vasodilation of most vascular beds (49). In addition, adenosine (via an A_{2A}AR) inhibits the aggregation of platelets and neutrophils and can thereby reduce a localized inflammatory response.

4. Adenosine and ischemic preconditioning

Ischemic preconditioning describes a process by which brief intermittent periods of ischemia provides protection against a more sustained ischemic episode. The cardioprotective action of ischemic preconditioning was described initially by Murry et al. (50) and has been supported by several other studies (for review, see reference 51). These investigators described a phenomenon that was relatively short-lived and diminished within a few hours. A second window of protection, evident about 24 h after the preconditioning treatment and associated with the synthesis of protective proteins, was subsequently described (52). Ischemic preconditioning of the myocardium has been observed in serveral different animal models including dogs (53), rabbits (54) and pigs (55) and also in humans (56, 57). It is believed that adenosine released during the preconditioning ischemic episodes is involved in the beneficial effects of preconditioning induced by either brief (58) or prolonged (59) ischemic episodes in the rabbit heart. However, the identity of the AR subtype mediating this action is still controversial. Various studies have implicated the A_1AR and the A_3AR in mediating ischemic preconditioning. Drugs which show selectivity for either the A_1AR or the A_3AR mimic the protection afforded by ischemic preconditioning (20, 60, 61). Overexpression of the A_1AR and A_3AR in chick cardiac myocytes confers protection against ischemia (62). However, given that the A_1AR exhibits a higher affinity for adenosine than the A_3AR, it is not clear to what extent the latter is activated during low-flow ischemia when the interstitial fluid adenosine levels are in the low micromolar range. Interestingly, while activation of the A_1AR is associated with significant negative chronotropic and dromotropic effects, activation of the A_3AR is devoid of these side effects (20). Since the negative chronotropic and dromotropic actions are unrelated to the cardioprotective effect of A_3AR agonists, these results suggest that drugs with A_3AR selectivity may prove more beneficial in patients. In contrast, activation of the A_{2A}AR abolishes the protective role of these receptors (60).

One mechanism underlying the AR-mediated preconditioning response involves activation of protein kinase C (63). This conclusion was derived indirectly, given that inhibition of protein kinase C during the preconditioning and prolonged ischemic phases blocked the beneficial response of preconditioning (63). Blockade of the preconditioning response was not reliably achieved but required a specific temporal sequence of inhibition of protein kinase C (64). There is no definitive evidence for membrane translocation of protein kinase C during ischemia and reperfusion. Simkhovich et al. (65) were unable to demonstrate actual translocation of this protein to the membrane fraction. However, transient membrane association of protein

kinase C with the plasma membrane has been demonstrated in the rat heart during ischemia-induced preconditioning (66) and following phorbol ester-mediated preconditioning response (67). Furthermore, ischemic preconditioning was associated with membrane translocation of the ε and η isoforms of protein kinase C (68). In a recent study, Liang (69) demonstrated that activation of the A_1AR in chick cardiac myocytes was involved in hypoxia-induced preconditioning response. Activation of the A_1AR stimulated protein kinase C and led to activation of an ATP-sensitive K^+ channel. The importance of these channels in the preconditioning response is inferred from the observation that inhibition by glibenclamide blocked protein kinase C-induced preconditioning response (69). Furthermore, direct activation of K_{ATP} channels by bimakalim (70) and cromakalin (71) led to reductions in infarct size in the dog heart. It was initially thought that the beneficial action of the K_{ATP} channel activators was due to shortening of the action potential duration. However, this appears unlikely since the protective action of these agents was observed even in quiescent cardiac myocytes which did not produce action potentials (72). A viable alternate explanation is that these K_{ATP} channel activators mediate their beneficial action by interacting with channels on the mitochondrial membrane instead of the sarcolemmal membrane (73). Activation of these K_{ATP} channels by protein kinase C likely involves phosphorylation of consensus sites on the channel protein (74, 75).

In addition to protein kinase C, the involvement of additional kinases in the development of preconditioning has been suggested. Administration of tyrosine kinase inhibitors blocked the ischemic preconditioning response in rat (76) and rabbit heart (77), implicating this kinase in the development of the phenomenon. Furthermore, adenosine increases the activity of p38 mitogen activated protein (MAP) kinase in cardiomyocytes (78, 79), while the activity of the MAPKAPK-2 is increased in the preconditioned heart. These latter results suggest an involvement of the MAP kinase pathway in ischemic preconditioning.

Since coupling of the A_3AR to phospholipase C-β and activation of protein kinase C in a rat basophilic leukemia clone was demonstrated previously (80, 81), a role of this receptor subtype in mediating the preconditioning response to adenosine was proposed. As described above, experimental evidence support an integral role of the A_3AR in mediating the ischemic preconditioning of cardiac myocytes in vitro, linked to the activation of protein kinase C (63, 69, 82, 83). Given the beneficial effects of antioxidants and antioxidant enzymes against ischemic reperfusion injuries (84), we tested whether adenosine can induce the activation of antioxidant enzymes. Activation of the A_3AR in the rat basophilic leukemia (RBL) cells led to a increase in the activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase, along with a reduction in malondialdehyde, a marker of lipid peroxidation (85) (Fig. 1). This stimulatory action appears to involve protein kinase C-mediated phosphorylation (86). Such a mechanism could serve to decrease the levels of ROS, which would otherwise be harmful to the cell. This effect of adenosine was also evident in vivo, and may account for adenosine-induced reduction of lipid peroxidation in the cochlea (87). Furthermore, the adenosine analog (*R*-PIA) has been shown to protect cochlear explants from oxidative damage induced by cisplatin (88) and confers protection against noise-induced loss of hair cells in the chinchilla cochlea (89).

Studies in the rat inferior colliculus, a central nucleus involved in processing of auditory signals, indicate that phosphorylation plays an integral role in the activation of these antioxidant enzymes. Incubation of inferior colliculus homogenates with alkaline phosphatase to induce dephosphorylation resulted in reductions in the activities of antioxidant enzymes, while subsequent incubation with protein kinase C restored enzyme activities (90). Restoration of enzyme activity was specific to the protein kinase C-α isozyme, but not to the β_1, β_2, δ or γ forms (Fig. 2). Taken together, these data may explain the mechanism underlying the beneficial role of A_3AR and protein kinase C activation in ischemic preconditioning.

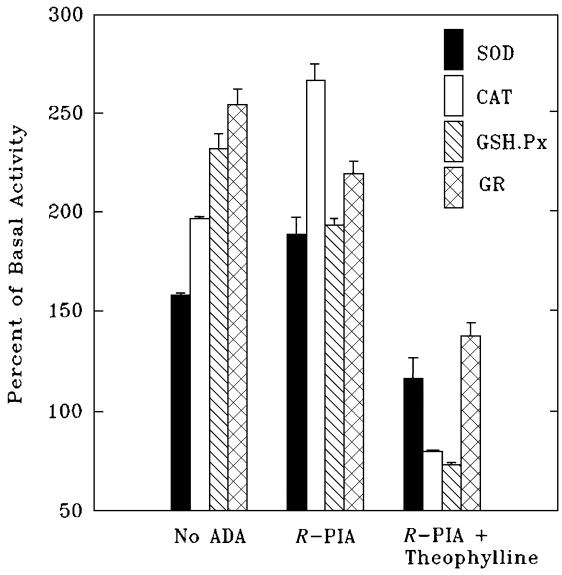

Fig. 1. Stimulation of the activity of antioxidant enzymes and glutathione reductase by adenosine analogs. Rat basophilic leukemia cells were treated with adenosine deaminase and further with *R*-PIA (10 μM) or *R*-PIA + theophylline (1 mM) for 90 min at 37°C. Basal activity of each enzyme was represented as the mean ± S.E.M. of 5 experiments, each performed using triplicate determinations. Increases in enzyme activity obtained in the no adenosine deaminase and *R*-PIA groups were statistically significant ($p<0.05$). (Reproduced from reference #85 with permission from Academic Press, Inc.)

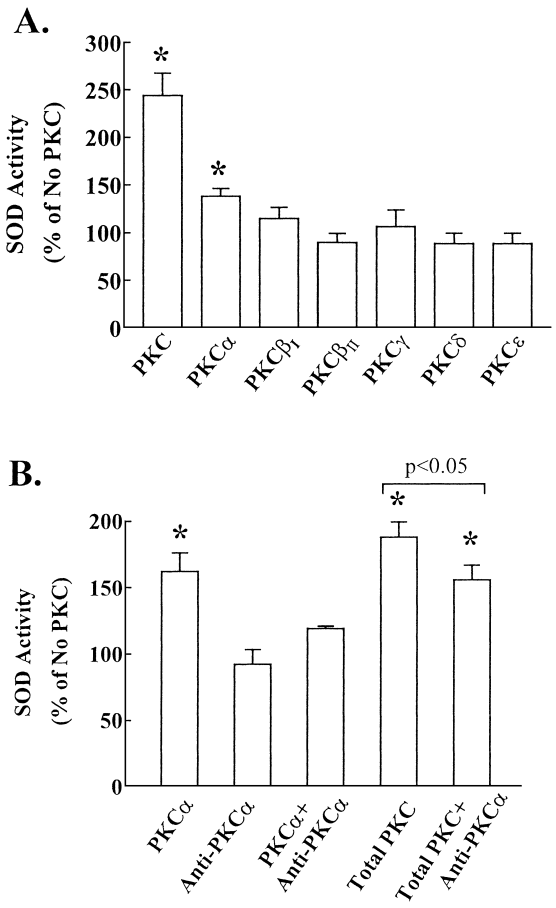

Fig. 2. Identity of the PKC isoform linked to the activation of superoxide dismutase. A: IC homogenates (from 3-month-old rats) were phosphorylated total rat brain protein kinase or an equivalent amount of the recombinant isoforms as described previously (90) and used for determining the activity of superoxide dismutase. Results are presented as the mean ± S.E.M. of three experiments, with asterisks indicating statistically significant difference from control ($p<0.05$). B: Monoclonal antibodies against protein kinase C-α blocked activation of superoxide dismutase. (Reproduced from reference #90 with permission from Elsevier Science)

5. Oxidative stress regulates A_1AR expression via activation of NFκB

While acute administration of adenosine increases the activities of antioxidant enzymes (as described above), we have recently demonstrated that the expression of the A_1AR subtype is regulated by oxidative stress (91). Oxidative stress induced by certain antineoplastic agents and by H_2O_2 up-regulates the A_1AR in hamster ductus deferens (DDT_1 MF-2) smooth muscle cells. Treatment of DDT_1 MF-2 cells with cisplatin, a chemotherapeutic agent which enhances ROS generation, increased the level of the A_1AR, determined by the binding of the antagonist radioligand 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), the the agonist radioligand ^{125}I-N^6-2-(4-amino-3-phenyl)ethyladenosine (APNEA) and by Western blotting, by approximately twofold (Fig. 3). Other inducers of reactive oxygen species, such as chemotherapeutic agents daunorubicin, doxorubicin and mitroxantrone and H_2O_2 were also effective in increasing the A_1AR (91). The clinically inactive platinum analog, transplatin, was ineffective in this regard. A role of reactive oxygen species generation in mediating the increase in A_1AR expression was supported by the finding that incubating cells with H_2O_2 and catalase, an scavenger of this reactive oxygen species, attenuated the response of H_2O_2. The increase in A_1AR was inhibited when cells were incubated with actinomycin D, suggesting a role for de novo receptor synthesis in mediating this process. Since certain chemotherapeutic agents, such as daunorubicin, can activate nuclear factor kappa B (NFκB) presumably through generation of reactive oxygen species (92), a role of NFκB in the induction of A_1AR was determined. In cells treated with cisplatin along with pyrrolidine dithiocarbamate or dexamethasone, inhibitors of NFκB activation (92), the induction of the A_1AR by cisplatin was attenuated (91). This finding suggests an obligatory role of NFκB in ROS-mediated increase in the A_1AR. A more direct demonstration of activation of the A_1AR gene by cisplatin was provided by the demonstration that this agent increases luciferase activity in cells transiently transfected with the A_1AR promoter (91), coupled to firefly luciferase reporter gene (Fig. 4). Activation of the A_1AR gene might account for cisplatin-induced up-regulation of the A_1AR in rat cochlea (93) and testis (94), in renal proximal kidney cell in cultures (95) and in primary cultures of rat embryonic neurons (D.M. Hallam et al., unpublished). We believe that up-regulation of the A_1AR in these tissues is an adaptative response to counter the oxidative stress. Thus, oxidative stress can enhance the purinergic system in two ways. First, oxidative stress promotes increases in extracellular adenosine release that can then activate ARs, leading to the enhancement of signaling pathways to provide cytoprotection. Second, oxidative stress itself induces expression of the A_1AR and possibly A_3AR (see below), which provide a more prolonged period of cytoprotection.

6. Role of NFκB in the regulation of both A_{2A}AR and A_3AR

In contrast to the A_1AR, oxidative stress decreased the expression of the A_{2A}AR. Treatment of rat pheochromocytoma (PC-12) cells with H_2O_2 resulted in a significant reduction in the expression of the A_{2A}AR, presumably mediated via activation of NFκB (96). Other activators of NFκB in these cells, such as ceramide and nerve growth factor (NGF), also inhibited A_{2A}AR expression. This effect of NGF was mediated via the low affinity (p75) NGF receptor and was blocked by inhibition of NFκB, implicating

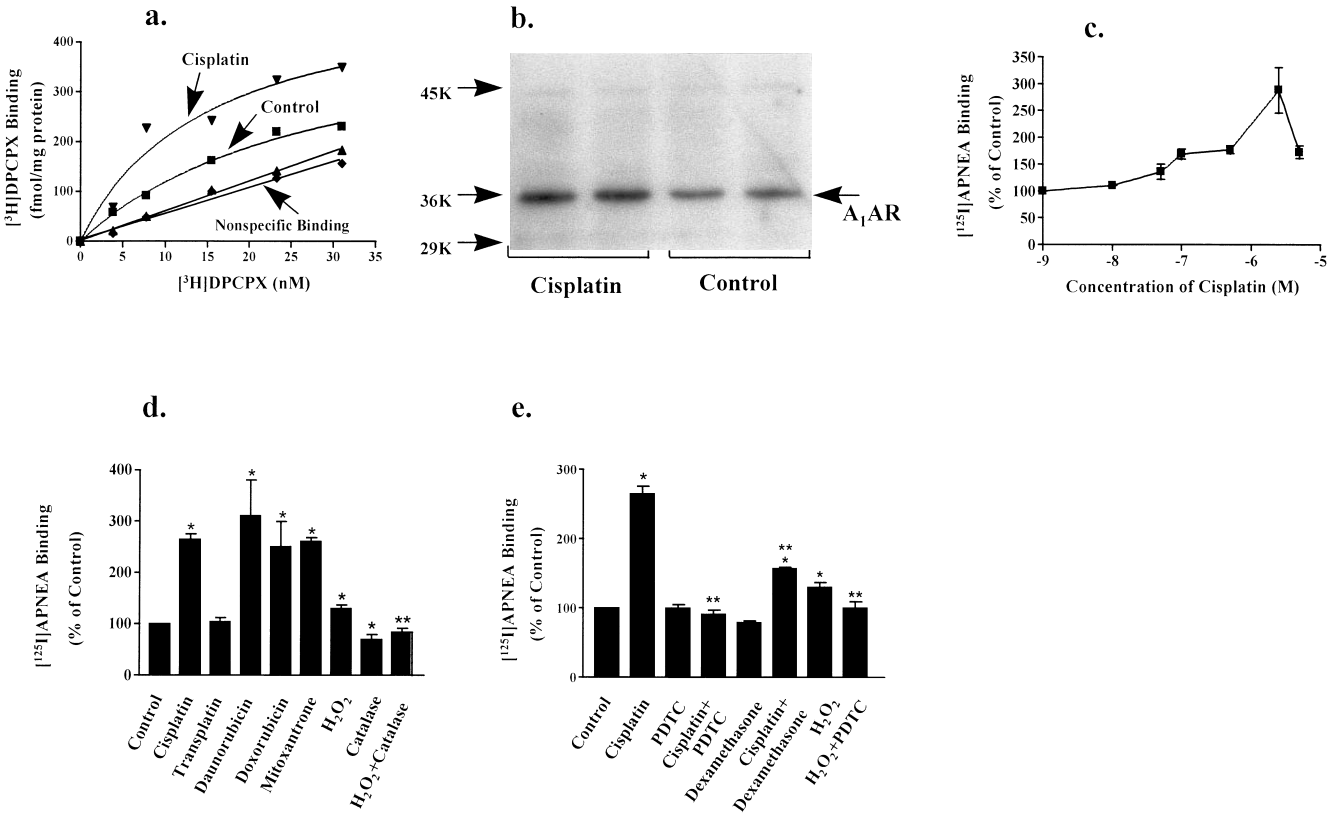

Fig. 3. Cisplatin treatment for 24 h induced up-regulation of the A_1AR in DDT_1MF-2 cells. Cells were treated with cisplatin (2.5 μM) for 24 h and crude plasma membranes were prepared for determining A_1AR. a, Saturation curves showing increase in A_1AR expression in DDT_1MF-2 cells after cisplatin treatment, as described by Nie et al. (91). b, Western blotting for the A_1AR using a polyclonal antisera at a titer of 1:1000. c, Dose-response effect of cisplatin obtained with cells treated with cisplatin for 24 h using ^{125}I-N^6-2-(4-amino-3-phenyl)ethyladenosine (1 nM). d, Up-regulation of the A_1AR by different chemotherapeutic agents and H_2O_2. Cells were incubated with 1 μM of the chemotherapeutic agents or 10 μM H_2O_2 or 200 units/ml catalase for 24 h. e, Inhibition of NFκB by pyrrodiline dithiocarbamate or dexamethasone. *, indicate statistically significant difference from control. **, statistical significant difference from the cisplatin-treated group. (Reproduced with permission from the American Society for Pharmacology and Experimental Therapeutics)

this transcription factor in the process of receptor down-regulation. However, the response to NGF was unaffected following blockade of the high affinity NGF receptor, TrkA, or following blockade of the MAP kinase pathway. Our working hypothesis concerning the regulation of the A_{2A}AR is that oxidative stress or NGF activates NFκB and then stimulates a consensus sequence for this factor in the upstream promoter region of the rat A_{2A}AR gene (97), and somehow mediates inhibition of transcription of this gene. The A_{2A}AR is not unique in this regard. NFκB-mediated inhibition of transcription of genes encoding the androgen receptor (98), the mouse *k* immunoglobulin light chain (99) and the major histocompatibility class II-invarient chain (100) has been demonstrated. One concern with the current hypothesis is that temporally, down-regulation of the A_{2A}AR preceded changes in its mRNA. For example, maximal change in A_{2A}AR was observed 24-h following NGF treatment, compared to a 3-day post-treatment requirement for optimal changes in mRNA. The explanation for this is not immediately apparent. However, these findings suggest that multiple mechanisms may explain down-regulation of the A_{2A}AR following activation of NFκB.

The A_3AR on rat mast mast cells plays an important role in augmenting histamine release following crosslinking of IgE. While the A_{2B}AR appears to be more important in degranulation of human mast cells (101), up-regulation of the A_3AR was observed on eosinophils of asthmatics (102). Since the asthmatic condition could be exacerbated by air pollution (containing a variety of reactive oxygen species) and concurrent airway inflammation, we investigated whether the A_3AR is also subjected to regulation by these stressors using a RBL clone. Oxidative stress, induced with hydrogen peroxide, produced a dose-dependent increase in A_3AR messenger RNA and protein (103). The increase in receptor protein was blocked by catalase and pyrrodiline dithiocarbamate, an inhibitor of NFκB, sug-

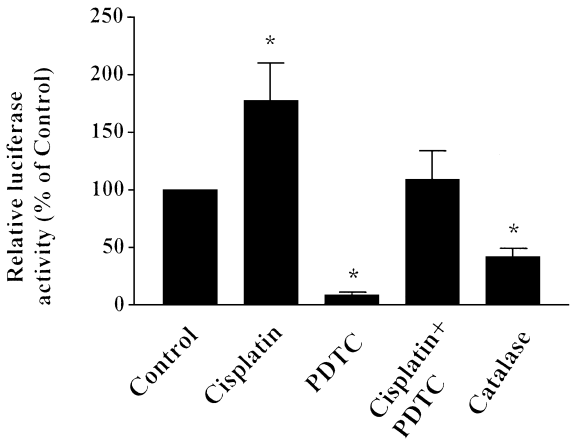

Fig. 4. Cisplatin-induced activation of luciferase activity. DDT_1MF-2 cells were transfected with plasmid (pBLPnif/PmtA) that contains the A_1AR promoter (promoter A) coupled to the firefly luciferase reporter gene (91). Luciferase activity was determined in controls or in cells exposed to cisplatin for 24 h in the absence or presence of pyrrodiline dithiocarbamate (200 μM) or catalase (200 units/ml). (Reproduced with permission from the American Society of Pharmacology and Experimental Therapeutics)

gesting an integral role of this transcription factor in the regulation of this receptor. Similarly, administration of tumor necrosis factor (TNF)-α, increased A_3AR expression. This effect was blocked by actinomycin D and cycloheximide, suggesting the involvement of gene transcription and protein synthesis, respectively. Treatment of RBL-2H3 cells with lipopolysaccharide and interferon-γ also up-regulated the A_3AR (103). Taken together, these data suggest that the A_3AR is subjected to regulation by oxidative stress and inflammatory mediators which are believed to activate NFκB. Such an increase in A_3AR on mast cells, coupled to an increase in A_1AR in the bronchioles may contribute to hypersensitivity of asthmatics to adenosine. In the cardiovascular system, however, up-regulation of the A_3AR might contribute to the ischemic preconditioning response.

7. Conclusion

In summary, adenosine plays an important role in mediating cytoprotection both in vitro and in vivo. This nucleoside can also contribute to the beneficial effect of ischemic preconditioning. Thus, drugs that mimic the actions of adenosine might be useful clinically in conditions such as stroke and myocardial infarction. A dynamic interaction appears to exist between the expression of AR subtypes and oxidative stress, mediated by NFκB and possibly other transcription factors. This finding suggests that in addition to oxidative stress, other activators of NFκB may promote cross-regulation of AR subtypes.

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