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Cisplatin up-regulates adenosine A_1 receptors in rat testes

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Abstract

Reactive oxygen species contribute to male infertility by reducing sperm function. Our laboratory has recently demonstrated that reactive oxygen species stimulate the expression of adenosine A_1 receptor which confers cytoprotection in a variety of tissues. Since the adenosine A_1 receptor is highly expressed in the testis, the goal of this study was to determine whether this testicular adenosine A_1 receptor could also be regulated in vivo by reactive oxygen species. Cisplatin, a chemotherapeutic agent shown to alter testicular function, was used to generate reactive oxygen species in vivo. Testes obtained from Sprague–Dawley rats treated with cisplatin (8 mg kg^{-1}) demonstrate increased lipid peroxidation and induction of heat shock protein by day 3. In addition, radioligand binding and Western blotting studies indicate an increase in testicular adenosine A_1 receptor in these rats. Scatchard analysis of [^{3}H]8-cyclopentyl-1,3-dipropylxanthine (DPCPX) binding data indicates a significant increase in adenosine A_1 receptor number (B_{max}) from 309 ± 77 to 540 ± 69 fmol mg^{-1} protein in the cisplatin-treated group. The respective equilibrium dissociation constants (K_ds) were 3.2 ± 1.5 and 3.0 ± 0.7 nM for the control and cisplatin-treated groups, respectively. Northern blotting analysis of rat testicular poly $(A)^+$ RNA indicates two adenosine A_1 receptor transcripts migrating at 3.4 and 5.6 kb, whose combined levels were increased by $49.3 \pm 9.3\%$ following cisplatin treatment. These results indicate that cisplatin enhances adenosine A_1 receptor expression in the rat testis, possibly through promotion of oxidative stress.

Keywords: Anticancer drug; Malondialdehyde; Oxidative stress; G protein-coupled receptor

1. Introduction

Adenosine mediates some of the physiological actions by interacting with four subtypes of adenosine receptors, namely the adenosine A_1, A_{2A}, A_{2B} and A_3 receptors. These receptors show differential distribution in rat testes. The adenosine A_1 receptor subtype is localized to the Sertoli cells, while the more abundant adenosine A_3 receptor is localized primarily to the germ cells (Rivkees, 1994). No adenosine A_2 receptor was detectable, despite a previous report suggesting the presence of this receptor on mouse sperms (Fraser and Duncan, 1993). In cultures of Sertoli cells, activation of adenosine A_1 receptor inhibits follicle-stimulating hormone (FSH)-mediated cyclic AMP production, the aromatization of androgen to estrogen (Meroni et al., 1998) and the secretion of pyruvate and inhibin (Conti et al., 1989). Activation of adenosine receptors inhibited FSH-mediated differentiation of Sertoli cells (Meroni et al., 1998). Antagonism of the adenosine receptors with caffeine alters sperm motility, respiration (Garbers et al., 1971), metabolism (Garbers et al., 1973) and its ability to penetrate the ovum (Schoenfeld et al., 1975).

It has been suggested that excessive generation of reactive oxygen species mediates injuries to the testes resulting from zinc deficiency (Oteiza et al., 1996), cryptorchidism (Zini and Schlegel, 1997) and following vasectomy (Aydos et al., 1998). Reactive oxygen species have also been implicated in male infertility, resulting from damage of sperm cells (Mazzilli et al., 1994). Protection from free radicals is mediated by an efficient free radical defense system, including antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase, and antioxidants such as ascorbic acid, tocopherols, glutathione and carotenes (Zini and Schlegel, 1997).

Recent studies have implicated reactive oxygen species in the toxicities associated with the use of a variety of chemotherapeutic agents. One such agent, cisplatin, has proven useful against a wide spectrum of human cancers, including bladder, head and neck, ovarian and testicular cancers. However, the use of this agent is limited by the development of nephrotoxicity and ototoxicity (Ward and

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Fauvie, 1976; Loehrer and Einhorn, 1984), toxicities which involve alterations in the antioxidant defense systems (Somani et al., 1995; Husain et al., 1996). Cisplatin has also been shown to alter the levels of luteinizing hormone (LH) and FSH, to reduce intratesticular testosterone, and to decrease sperm motility and count (Seethalakshmi et al., 1992; Aydiner et al., 1997). Histological examination of these testes further indicates significant damage to Sertoli, Leydig and germ cell populations induced by cisplatin (Aydiner et al., 1997).

Our laboratory has recently shown a link between the oxidative stress induced by cisplatin and the induction of the adenosine A_1 receptor. The goal of the present study was to determine whether the testis undergoes oxidative stress following cisplatin treatment and whether this is associated with up-regulation of the adenosine A_1 receptor.

2. Methods

2.1. Animals and sample collection

Animals used in this study were male Sprague–Dawley rats weighing 250–275 g. These were obtained from Harlan Laboratories (Indianapolis, IN) and were maintained on pulverized regular food and water and were housed in temperature-controlled rooms with 12 h light/dark cycle. The animals were kept in the metabolic cages that allowed separation of urine and feces. The care and use of animals reported in this study were approved by Southern Illinois University, School of Medicine Animal Care and Use Committee.

To see the effect of cisplatin, each rat was anesthetized with intramuscular injection of xylazine/ketamine (5.5 mg kg^{-1} xylazine and 17.2 mg kg^{-1} ketamine). Sterile saline (0.9% w/v NaCl) or cisplatin (8 mg kg^{-1} body weight) was infused over a period of 30 min. Animals were sacrificed 3 days following cisplatin treatment and testes were rapidly dissected free of epididymis and frozen in liquid nitrogen for radioligand binding, Northern blotting and Western blotting assays.

2.2. Membrane preparation

The rat testicular membranes were prepared exactly as previously described (Stiles et al., 1986). In brief, frozen testes were thawed and placed in ice-cold 50 mM Tris–HCl (pH 7.4) containing 10 mM $MgCl_2$, 1 mM EDTA, 10 μg ml^{-1} soybean trypsin inhibitor, 10 μg ml^{-1} benzamidine and 2 μg ml^{-1} pepstatin (buffer A). The tissue was then homogenized by Polytron (Brinkmann, setting 7) for 40 s at 4°C. Following centrifugation at $1000 \times g$ for 10 min, the supernatant was centrifuged at $40{,}000 \times g$ for 15 min. The resulting pellet was suspended in buffer A to give a final protein concentration of 1 mg ml^{-1}. Prior to performing radioligand binding assays , crude plasma membrane preparations were incubated with adenosine deaminase (5 U ml^{-1}) at 37°C for 10 min in order to eliminate endogenous adenosine.

2.3. Radioligand binding assay

The levels of adenosine A_1 receptor in rat testicular membranes were determined using the selective antagonist, [^{3}H]8-cyclopentyl-1,3-dipropylxanthine ([^{3}H]DPCPX). The assays were performed by incubating membranes (75 μg protein) at 37°C for 1 h with various concentrations of [^{3}H]DPCPX in the absence (total binding) or presence (non-specific binding) of theophylline (0.5 mM) in a total volume of 250 μl of buffer A. Non-specific binding ranged from 20% to 30% of total binding. After incubation, samples were filtered through GF/B glass fiber filters using a cell harvester (Brandel, Gaithersburg, MD) and quickly washed with 9 ml of ice-cold buffer A containing 0.01% 3-[(3-cholamidorpropyl)-dimethylammonio]-1-propanesulfonate (CHAPS). Bound radioactivity was determined using a liquid scintillation counter. Saturation curves and competition curves were analysed by a computer-based curve fitting program (Graph Pad PRISM, San Diego, CA).

2.4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting

Testicular membranes were solubilized in SDS-PAGE buffer at a concentration of 2 μg protein per microliter. Samples (75 μg) were electrophoresed by SDS-PAGE according to the method of Laemmli (1970). The proteins were then transferred to nitrocellulose filters using a Nova Blot apparatus (Pharmacia Biotect, Piscataway, NJ), blocked in blotto solution (130 mM NaCl, 2.7 mM KCl, 1.8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 0.1%NaN_3, and 5% low-fat skim milk) containing 0.1% Triton X-100 and incubated at 4°C overnight with G protein antibodies (Raymond et al., 1993; Gettys et al., 1994). These antibodies were obtained from Dr. Tom Gettys, Medical University of South Carolina, Charleston, SC. Following incubation, the blots were washed five times (10 min each) with blotto solution and incubated with ^{125}I-labelled goat anti-rabbit IgG (300,000 cpm ml^{-1}) for 1 h at room temperature. Blots were then washed five times (10 min each) in blotto containing 1% Triton X-100 prior to exposing them to autoradiographic films.

The levels of adenosine A_1 receptor were also determined by Western blotting using a polyclonal antiserum (purchased from Alpha Diagnostic International, San Antonio, TX). Testicular membranes were gently homogenized on ice in buffer A containing CHAPS, with a CHAPS-to-protein ratio of 2.5:1. Samples were then stirred on ice for 1 h and centrifuged at $40{,}000 \times g$ for 15 min. The supernatants were desalted on Sephadex G-25 column and

equilibrated with buffer A containing 0.1% CHAPS. The eluates (100 μg protein each) were solubilized in SDS-PAGE buffer, resolved by SDS-PAGE and used in Western blotting studies.

The levels of heat shock protein (Hsp) 70 and 72 were determined by Western blotting studies using monoclonal antibodies for Hsp70 (Sigma, St. Louis, MO) and Hsp72 (Amersham Life Science, Arlington Heights, IL) and Enhanced Chemiluminescence Horse Radish Peroxidase detection Kit (Amersham Life Science) according to manufacturer's protocol. Twenty-five and 100 μg of cytosolic proteins were used for estimation of Hsp70 and Hsp72, respectively.

Quantitation of adenosine A_1 receptor, G proteins, Hsp70 and Hsp72, was performed by exposing the blots to phosphor imager screens, followed by densitometric scanning of the image using a GS-250 Molecular Imager (Bio-Rad, Hercules, CA).

2.5. RNA preparation and Northern blotting

Isolation of total RNA was performed using triazol reagent kit (Gibco BRL), and selection of poly $(A)^+$ messenger RNA using oligo-dT cellulose was performed as described previously (Davis et al., 1986). For Northern blotting experiments, poly $(A)^+$ RNA samples (10 μg)

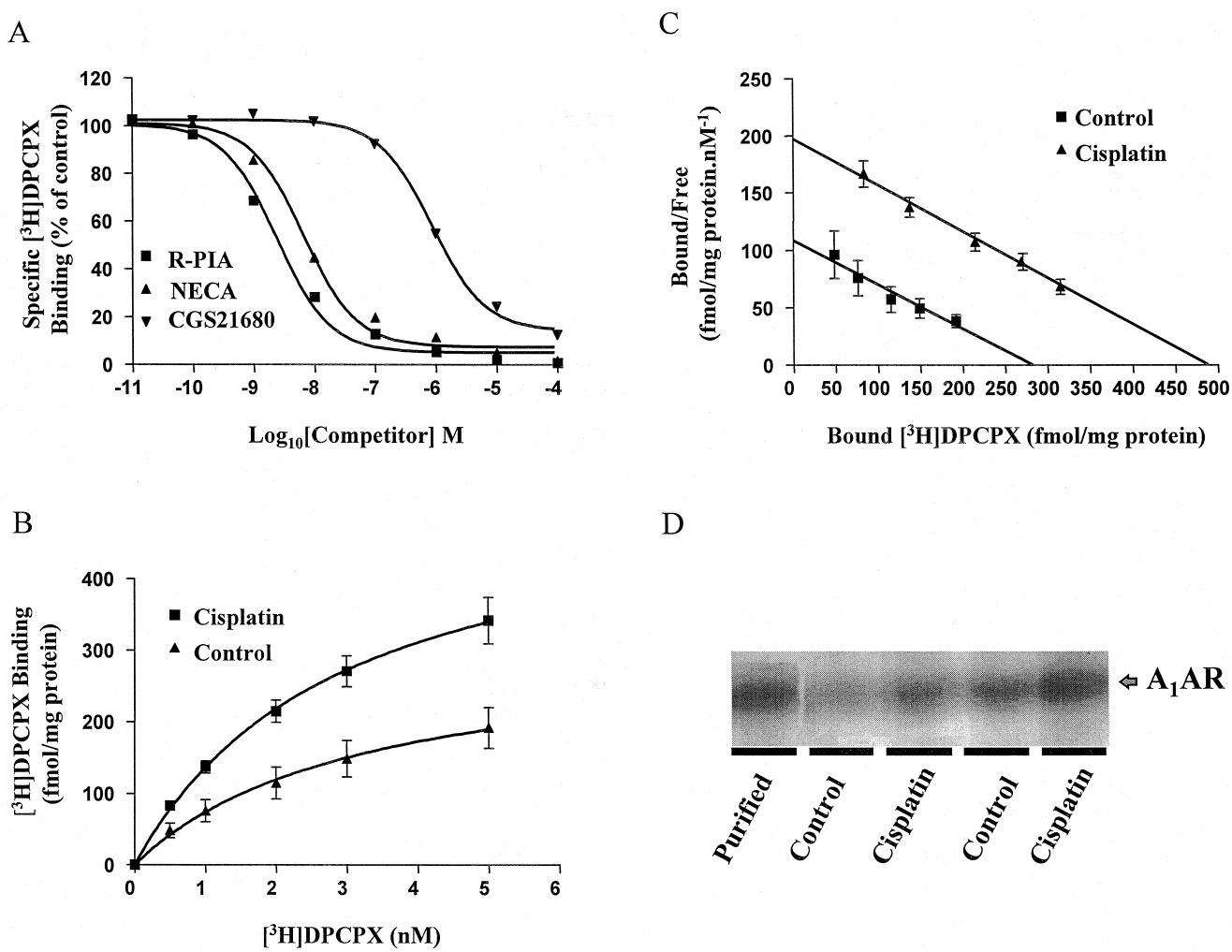

Fig. 1. Cisplatin up-regulates the adenosine A_1 receptor in rat testis. (A) Identification of the adenosine A_1 receptor subtype in rat testis using [^{3}H]DPCPX in agonist competition experiments. [^{3}H]DPCPX was incubated with membrane protein (75 μg) and various competitors. Competition curves were generated using GraphPad Prism software. The respective K_i values were 1.4×10^{-9}, 4.7×10^{-9} and 7.2×10^{-7} M for *R*-PIA, NECA and CGS21680, respectively. (B) Saturation binding experiments were performed by incubating testicular membranes with [^{3}H]DPCPX in the absence (total binding) or presence of 0.5 mM theophylline (nonspecific binding) for 1 h at 37°C. Curves, representing specific binding, were fitted using a one-site model by the Graph Pad PRISM software. Data are presented as the mean ± S.E.M. of three animals. (C) Scatchard transformation of the data in (B). (D) Quantitation of changes in adenosine A_1 receptor expression in testes by Western blotting. Testicular membranes were resolved on a 12% acrylamide gel. The adenosine A_1 receptor antibody (Alpha Diagnostic International, San Antonio, TX) was used at a titer of 1:1000. The adenosine A_1 receptor bands were visualized using ^{125}I-labeled goat anti-rabbit IgG (300,000 cpm ml^{-1}). Purified testicular adenosine A_1 receptor (50 fmol), obtained by affinity chromatography, served as a control marker. Cisplatin treatment increased adenosine A_1 receptor immunoreactivity by 55.5 ± 8.1 % over control (mean ± S.E.M. of four rats).

were electrophoresed on 1% agarose/MOPS/formaldehyde gel, transferred to nitrocellulose membrane, and cross-linked in Stratagene UV cross-linker. Prehybridization mixture contained 5 × SSC (1 × SSC contains 0.15 M sodium chloride and 0.015 M sodium citrate, pH 7.0), 2 × Denhardt's (1 × contains 0.2 g l^{-1} (w/v) each of polyvinylpyrolidine, bovine serum albumin and ficoll), 1% SDS, 0.2 mg ml^{-1} salmon sperm DNA, and 50% formamide. Hybridization mixture (10 ml) was essentially the same except that the Denhardt's concentration was 2.5 × and the mixture contained ^{32}P-labelled canine cDNA probes encoding the adenosine A_1 receptor at concentration of $1–2 \times 10^6$ cpm ml^{-1}. Hybridizations were performed by shaking blots in a 42°C water bath for 16–24 h. Following incubations, blots were washed twice (15 min each) at room temperature in 2 × SSC and 0.1% SDS and twice (20 min each) with 0.1 × SSC and 0.1% SDS at 62°C. Blots were then subjected to autoradiography for 1–4 days. Northern blots were normalized using a labelled cDNA probe encoding the human GAPDH. The relative band intensities were determined by densitometric scanning on the GS-250 Molecular Imager after exposing the blots to imager screen for 6–12 h.

2.6. Malondialdehyde assay

The assay method used is based on that described by Ohkawa et al. (1979). Tissue homogenates (200 μl) were incubated with 50 μl of 8.1% sodium dodecyl sulfate at room temperature for 10 min. Following incubation, 375 μl of 20% acetic acid (pH 3.5) and 375 μl of 0.8% thiobarbituric acid (in 0.5 N NaOH) were added and the mixture was boiled for 1 h. The mixture was then cooled and extracted with 1 ml each of *n*-butanol–pyridine mixture (1:3, v/v) to avoid turbidity. The upper layer (approximately 0.6 ml) of each sample was aspirated and the absorbance measured at 532 nm. Concentrations of 2-thiobarbituric acid were determined using the extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}.

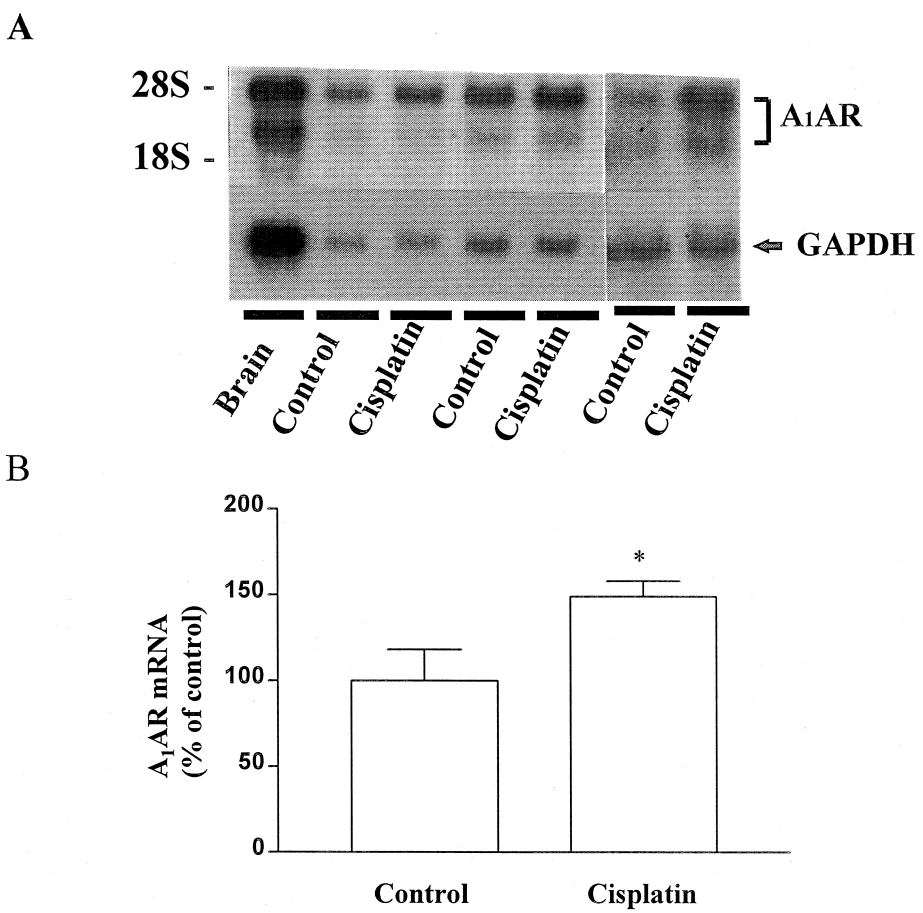

Fig. 2. Cisplatin-increased the steady state levels of adenosine A_1 receptor mRNA in rat testis. A, Poly $(A)^+$ RNA (10 μg), obtained from control rats and cisplatin-treated rats were used in Northern blotting assays. Blots were probed with a ^{32}P-labeled canine adenosine A_1 receptor cDNA at concentration of $1–2 \times 10^6$ cpm ml^{-1} of hybridization solution. Two adenosine A_1 receptor transcripts of 5.6 and 3.4 kb were detected and the band intensity for each treatment was quantitated using a phosphor imager. Blots were normalized by first stripping them and reprobing with a labeled human GAPDH cDNA. (B) Graphical representation of the phosphor imager data in (A). The data are presented as the mean ± S.E.M. of four animals. Asterisk indicates statistically significant difference ($P < 0.05$) from control.

2.7. Cell culture

DDT_1 MF-2 cells were cultured in monolayers in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 5% calf serum. Cells were treated with either testosterone (50 nM) or β-estradiol (1 μM) for 24 h and then used for radioligand binding studies.

2.8. Statistical analysis

Statistical differences among means were determined using Student's *t*-test or by analysis of variance (ANOVA) followed by Tukey's post-hoc analyses.

3. Results

3.1. Cisplatin increases adenosine A_1 receptor expression in rat testis

To determine whether the receptor identified by antagonist [^{3}H]DPCPX in the testes was the adenosine A_1 receptor, agonist competition curves were performed. Competition curves indicate that the order of potency of adenosine analogs was *R*-phenylisopropyladenosine (*R*-PIA) > *N*-ethylcarboxamide adenosine (NECA) > 2-[*p*-(2-carboxyethyl)phenylethyl amino]5′-*N*-ethylcarboxamidoadenosine (CGS21680), suggesting that these binding sites were likely not the adenosine A_{2A} receptor (Fig. 1A). The inhibition constants (K_i) for these agonists were, respectively, 1.4×10^{-9}, 4.8×10^{-9} and 7.6×10^{-7} M. The high affinity of *R*-PIA and NECA for these sites is indicative of interaction of these analogs with the adenosine A_1 receptor and not the adenosine A_3 receptor.

Saturation plots, performed using the antagonist radioligand [^{3}H]DPCPX, indicate up-regulation of adenosine A_1 receptor in the testis by cisplatin. The specific binding obtained was 68.5 ± 5.1% of total binding at concentration of [^{3}H]DPCPX around the equilibrium dissociation constant (K_d) (Fig. 1B). Scatchard representation of the data, shown in Fig. 1C, indicates cisplatin-induced increase in

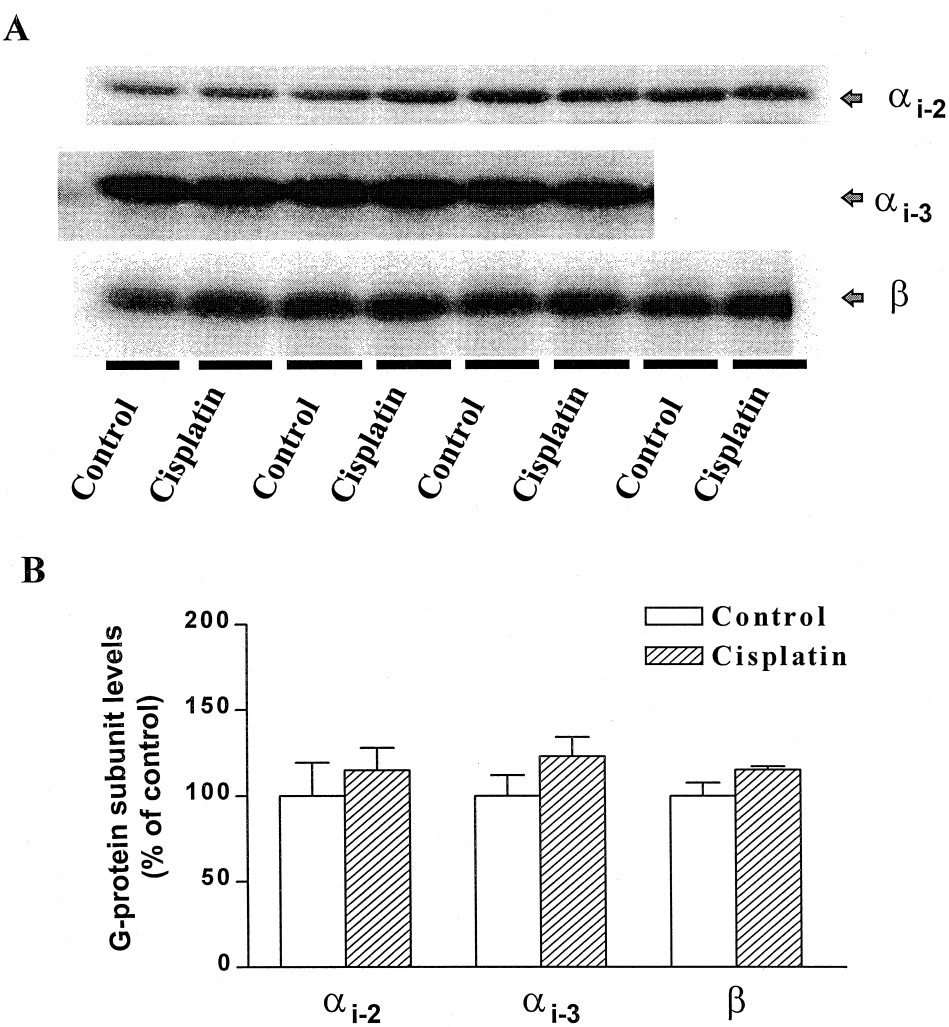

Fig. 3. Regulation of G_i protein subunit expression in rat testis by cisplatin. Testicular membrane protein (75 μg) was solubilized and electrophoresed on SDS-PAGE (12% acrylamide gels). (A) Western blotting was performed essentially as described in Section 2 using polyclonal antibodies against G_i protein subunits. The relative intensities of the bands were determined using a phosphor imager. (B) Graphical representation of the data (mean ± S.E.M.) obtained in (A).

total receptor number (B_{max}) without change in K_d. The B_{max} values estimated from testicular membranes were 309 ± 77 and 540 ± 69 fmol mg^{-1} protein (mean ± S.E.M.) for control and cisplatin-treated rats, respectively. This increase in the cisplatin-treated groups was statistically significant from the control group ($P < 0.05$). The respective K_d values were 3.2 ± 1.5 and 3.0 ± 0.7 nM, for the control and cisplatin-treated animals, respectively.

R-PIA competition curves were performed, using [^{3}H]DPCPX as the radioligand, to determine whether cisplatin treatment also altered the different affinity states of the adenosine A_1 receptor. These competition curves were best fitted by a two-state model with the high- and low-affinity K_i values for the controls being $9.4 \pm 1.8 \times 10^{-10}$ and $1.4 \pm 0.7 \times 10^{-7}$ nM, respectively, and with $82 \pm 6\%$ of the receptors in the high-affinity state. No significant changes in the high- and low-affinity K_i values or percentage of receptors in these states were observed in the cisplatin-treated animals. These values were $1.5 \pm 0.3 \times 10^{-9}$ and $1.6 \pm 1.1 \times 10^{-7}$ nM, respectively, with $77 \pm 8\%$ of the receptors being in the high-affinity state.

Western blotting studies for the adenosine A_1 receptor indicate labeling of a 42-kDa protein in both testicular membranes and affinity-purified rat testicular adenosine A_1 receptor, suggesting that the labeled membrane protein is likely the adenosine A_1 receptor. Compared to testicular preparations from the control animals, the levels of adenosine A_1 receptor in the cisplatin-treated animals were increased by $55.5 \pm 8.1\%$ (Fig. 1D). These values were statistically significant from those of the control animals ($P < 0.05$).

3.2. Cisplatin increases adenosine A_1 receptor mRNA in rat testis

Due to the low levels of adenosine A_1 receptor mRNA, detection on Northern blots was performed on poly $(A)^+$ RNA preparations. Blots were hybridized with a labeled cDNA probe derived from the canine adenosine A_1 receptor (Libert et al., 1991). Two adenosine A_1 receptor transcripts of 3.4 and 5.6 kb were detected (Fig. 2A). Blots

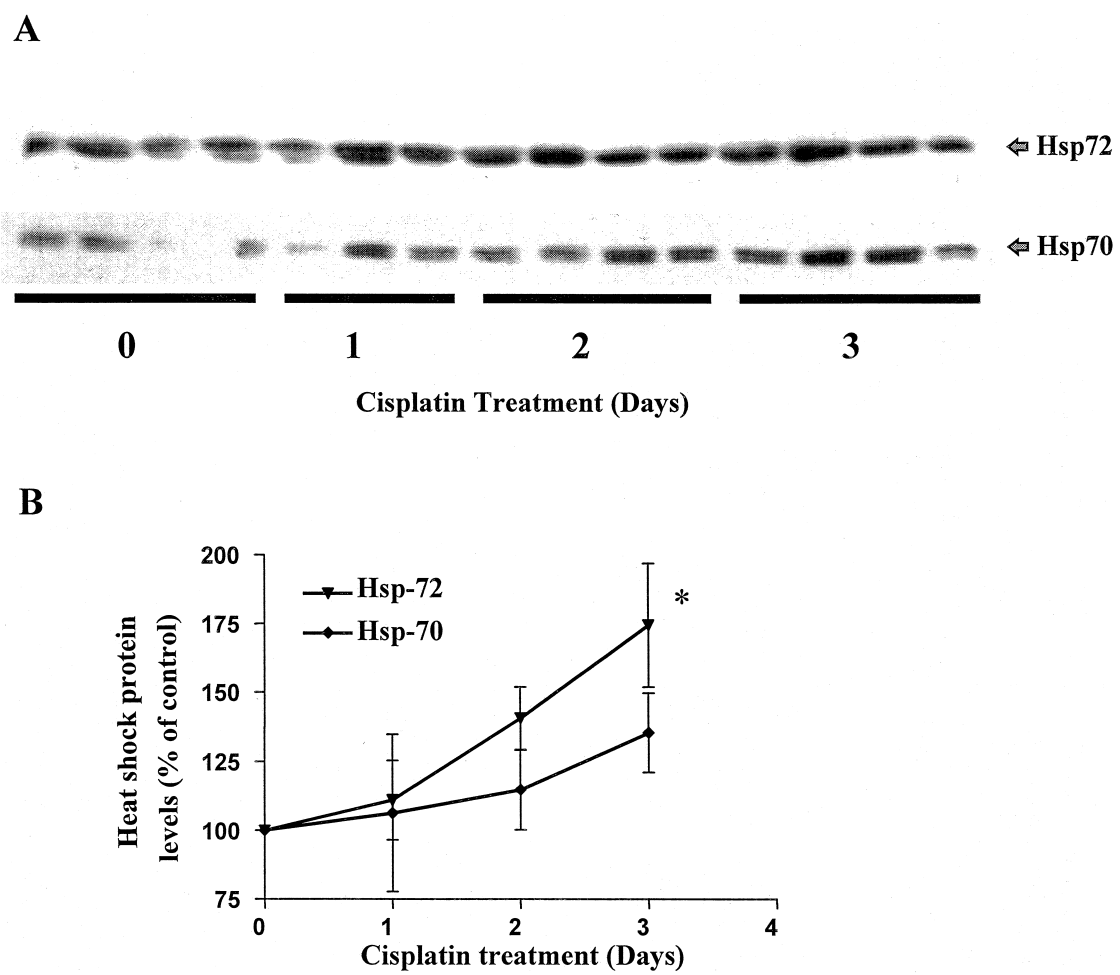

Fig. 4. Cisplatin induces heat shock protein expression in rat testis. (A) Western blots were performed with testicular cytosols (50 μg), following different durations of cisplatin treatment, using monoclonal antisera against Hsp70 and Hsp72 (1:1000 titer). Blots were visualized using Enhanced Chemiluminescence and quantitated with a phosphor imager. (B) Graphical representation of cisplatin-induced Hsp70 and Hsp72 expression in the rat testis, showing the mean ± S.E.M. of four animals from each time point. Asterisk indicates statistically significant difference ($P < 0.05$) from control.

were then stripped and reprobed with a cDNA encoding the human glyceraldehyde phosphate dehydrogenase (GAPDH) for normalization. Following normalization to GAPDH mRNA, the steady-state levels of adenosine A_1 receptor mRNA in the cisplatin-treated animals increased by $49 \pm 9\%$ over that of the control animals (Fig. 2B).

3.3. Regulation of G_i protein expression by cisplatin

To determine whether up-regulation of the adenosine A_1 receptor was accompanied by increases in G_i coupling proteins, Western blotting studies were performed for G protein subunits. The levels of α_{i2}, α_{i3} and β were all unchanged following cisplatin treatment (Fig. 3A,B). The relative levels of α_{i2}, α_{i3} and β subunits in the cisplatin-treated group were, respectively, $117 \pm 13\%$, $123 \pm 11\%$ and $115 \pm 2\%$ of control.

3.4. Increase in lipid peroxidation in rat testis by cisplatin

Since up-regulation of the adenosine A_1 receptor by cisplatin was associated with increased generation of reactive oxygen species (Nie et al., 1998), we tested the possibility that the increase in adenosine A_1 receptor in the rat testes was also associated with an increase in reactive oxygen species. The effect of reactive oxygen species was determined indirectly by assaying for malondialdehyde, a marker of lipid peroxidation. Malondialdehyde levels were significantly increased ($P < 0.05$) by $57.4 \pm 8.2\%$ in rat testis following cisplatin treatment for 3 days. The actual levels of malondialdehyde in the testes were 0.61 ± 0.07 and 0.96 ± 0.05 nmol mg^{-1} protein for control and cisplatin-treated rats, respectively.

3.5. Cisplatin increased heat shock protein expression in rat testis

To further determine whether in vivo cisplatin treatment could induce stress to the testes, we measured the expression of Hsp70 family of proteins which are known to be induced by stress (Essig and Nosek, 1997; Mayer and Bukau, 1998). Quantitation of the constitutive Hsp70 expression indicates a $35 \pm 14\%$ increase following cisplatin treatment for 3 days. Hsp72 was expressed in vivo in the testes in the absence of cisplatin, albeit at lower levels than Hsp70. In the presence of cisplatin, Hsp72 expression was time-dependently regulated, being increased by $11 \pm 16\%$, $60 \pm 11\%$ and $74 \pm 23\%$, following cisplatin treatment for 1, 2 and 3 days, respectively (Fig. 4).

3.6. Testosterone and β-estradiol treatment did not alter adenosine A_1 receptor expression in DDT_1 MF-2 cells

To determine whether the effect of cisplatin was produced secondarily to reductions in testosterone levels (Seethalakshmi et al., 1992), we tested the ability of testosterone to regulate adenosine A_1 receptor expression. Incubation of DDT_1 MF-2 cells with testosterone for 24 h produced no change in adenosine A_1 receptor, discounting a role of this hormone in mediating the response of cisplatin. Similarly, no significant change in adenosine A_1 receptor expression was obtained following exposure of these cells to β-estradiol for 24 h.

4. Discussion

This study demonstrates up-regulation of adenosine A_1 receptor in the rat testis by cisplatin. The increase in adenosine A_1 receptor was associated with increase in the steady-state levels of adenosine A_1 receptor mRNA and increased oxidative stress. This in vivo regulation of the adenosine A_1 receptor mimics the in vitro effect of cisplatin observed in cell culture (Nie et al., 1998). In this latter study, oxidative stress appeared critical in mediating adenosine A_1 receptor up-regulation.

The adenosine receptor binding sites labeled by [^{3}H]DPCPX are presumed to be the adenosine A_1 receptor subtype, since this radioligand is a selective adenosine A_1 receptor antagonist. Furthermore, agonist competition curves indicate a potency rank of *R*-PIA > NECA > CGS21680, indicative of the labeled sites being the adenosine A_1 receptor (Fig. 1A). The estimated B_{max} obtained with this radioligand was comparable to those obtained previously in rat testis (Bhat et al., 1998). While the localization of the adenosine A_1 receptor in rat testes was not performed in this study, previous studies have localized these receptors within the seminiferous tubules (Murphy et al., 1983) and Sertoli cells (Rivkees, 1994). The rat testis also expresses a high concentration of adenosine A_3 receptors which are also localized to the seminiferous tubules (Murphy et al., 1983), germ cells and Leydig cells (Rivkees, 1994).

Two pieces of evidence from this study suggest that the testis experiences oxidative stress following cisplatin treatment. First, cisplatin increased the level of lipid peroxidation, as determined by elevations in malondialdehyde levels. Increases in malondialdehyde levels in the testis likely reflect the consequence of increased generation of reactive oxygen species by cisplatin. Alterations in the activities of antioxidant enzymes in other tissues and increases in malondialdehyde levels have also been observed following cisplatin administration in rats by other investigators (Somani et al., 1995; Husain et al., 1996). Second, cisplatin increases the expression of the stress protein, Hsp72. The expression of this protein is known to be regulated under a variety of stress conditions, including oxidative stress (Mayer and Bukau, 1998). We therefore propose that the in vivo up-regulation of adenosine A_1 receptor results from an increase in oxidative stress induced by cisplatin. One established pathway triggered by oxidative stress in-

volves activation of nuclear factor (NF) κB and activation of NFκB-regulated genes. One such gene that appears to be critically dependent on this transcription factor is the adenosine A_1 receptor (Nie et al., 1998).

In addition to the proposed mechanism involving activation of NFκB, other mechanisms for regulating adenosine A_1 receptor expression may also be possible. Cisplatin could activate other transcription factors, such as activating protein (AP)-1, which are present in the adenosine A_1 receptor gene promoter (Ren and Stiles, 1995). Furthermore, one possible action of cisplatin which we are currently investigating is whether this drug blocks the normal process of internalization of these receptors from the cell surface. Such an action would result in a net increase in adenosine A_1 receptors on the cell surface.

ROS-mediated up-regulation of adenosine A_1 receptor may play a critical role in facilitating cytoprotection in response to tissue injury as may occur during ischemia (Rudolphi et al., 1989). In vivo manipulations of adenosine A_1 receptor expression in the brain have been shown to modulate the susceptibility of the brain to ischemia (von Lubitz et al., 1994). As such, up-regulation of the adenosine A_1 receptor would likely subserve a protective role over a more delayed time period. Significant damage to Sertoli cells has also been detected following cisplatin treatment. Since these cells are the primary source of the adenosine A_1 receptor in the testes, up-regulation of the adenosine A_1 receptor by cisplatin may indirectly support a role of these receptors in cytoprotection.

It is not clear whether an additional mechanism underlying cisplatin-mediated regulation of adenosine A_1 receptor expression might not involve sex steroids. Cisplatin has been shown to decrease testosterone synthesis by Leydig cells (Seethalakshmi et al., 1992; Aydiner et al., 1997), likely resulting in positive feedback increases in FSH and LH levels in rats treated with cisplatin (Maines et al., 1990; Aydiner et al., 1997). Whether testosterone acts in vivo to inhibit adenosine A_1 receptor expression is not clear at present. In DDT_1 MF-2 cell cultures, at least, testosterone did not alter the expression of the adenosine A_1 receptor. Thus, it is possible that increase in oxidative stress might be the principal contributor to cisplatin-mediated adenosine A_1 receptor up-regulation in the testes.

Increase in the inducible Hsp72 protein may underscore another cytoprotective mechanism induced by cisplatin. In general, heat shock proteins help maintain cellular protein conformation during stress conditions, an action which complements preexisting antioxidant pathways (Essig and Nosek, 1997). A possible role of heat shock proteins is to help maintain the native confirmation of newly synthesized adenosine A_1 receptor following release from the Golgi apparatus, leading to a higher percentage of receptors reaching the cell membrane. In human glioblastoma cells, cisplatin increases Hsp72 levels by threefold (Matsumoto et al., 1996). In the cardiovascular system, this protein is induced by oxidative stress, ischemia, or hyperthermia and mediates cytoprotective and cellular repair functions (Mayer and Bukau, 1998).

Short-term and long-term adverse effects of acute and chronic cisplatin treatment on testicular functions have previously been documented in the rat, mouse and in human. In mice, Vawda and Davies (1986) showed decrease in resting primary spermatocytes, sperm motility and testicular DNA synthesis, which became significant by 3 days of cisplatin administration. In Sertoli and Leydig cells, a single dose of cisplatin produced dilation of endoplasmic reticulum, increase in lipid and lipofusion inclusions, disorganization of mitochondrial structure and distortion in the germ cells (Aydiner et al., 1997). Chronic cisplatin treatment reduced the number of spermatogonia and may lead to sterility (Loehrer and Einhorn, 1984).

In summary, the present study provides evidence of cisplatin-induced adenosine A_1 receptor expression in vivo, presumably in response to oxidative stress. Increase in adenosine A_1 receptor may subserve a cytoprotective role to mitigate the extent of cisplatin toxicity. As such, agonists of this adenosine receptor subtype or antioxidants may be useful protective agents against cisplatin-induced testicular damage.

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