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Reactive oxygen species mediate dopamine-induced signaling in renal proximal tubule cells

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ABSTRACT

Intrarenally-produced dopamine (DA) induces a large increase in urinary sodium excretion mainly due to the inhibition of tubular sodium reabsorption. We aimed to study the participation of reactive oxygen species (ROS) in DA signaling pathway in proximal tubule cells. Our results show that DA increased ROS production in OK cells and indicate the mitochondria as the main source of ROS. DA also increased ERK1/2, superoxide dismutase (SOD) and transcription factor κB (NF-κB) activity. These findings suggest that DA generates mitochondria-derived ROS that activate ERK1/2 and subsequently NF-κB and SOD activity at concentrations that exert a physiological regulation of renal function.

1. Introduction

Dopamine (DA) is a biologically active catecholamine which, apart from its established neurotransmitter role, regulates a variety of cellular functions. Physiological effects of DA influence renal and intestinal sodium and water homeostasis [1,2], vascular dynamics [3] and lung alveolar function [4] among others. The renal dopamine system plays an important role in the regulation of sodium excretion and blood pressure. Numerous studies show that DA exerts a natriuretic effect mainly dependent on the inhibition of both proximal and distal tubule sodium reabsorption.

DA is metabolized via monoaminoxidase (MAO) to 3,4-dihydroxyphenylacetic acid, a reaction that generates H_2O_2, but it can also be rapidly oxidized either spontaneously or enzymatically to DA semiquinone/quinone which form thiol conjugates with sulfhydryl groups on cysteine, glutathione and proteins exhibiting potent oxidizing properties [5]. Oxidized DA, through redox cycling [6], can also generate H_2O_2 and O_2^-, which are known to evoke lipid, protein and DNA modifications.

Evidence of DA-induced cellular damage has accumulated mainly from studies in the central nervous system [7–10] and a number of investigations point to DA and its metabolites as key contributors of ROS-induced cell damage in neurodegeneration and Parkinson's disease [11,12]. Moreover, ROS have also been syndicated in the development of hypertension [13] and oxidative stress during hypertensive states is known to impair the effects of DA within the kidney [14]. This is consistent with the notion that high levels of ROS are generated during the course of many pathological conditions and that they play a causative role in the etiology of a diverse array of diseases. Low levels of ROS, however, are generated in a highly controlled manner by various cell types to act as cellular messengers in redox-sensitive pathways and a growing body of evidence challenges the idea of ROS as mere intermediaries of disease [15]. In view of its potential to generate ROS, DA regulation of water and electrolyte transport within the kidney should involve minute amounts of those species and/or invoke an adequate antioxidant response in order to avoid damage to the cell environment.

Evidence indicates that ROS mediate the activation of MAP kinases (MAPKs) in multiple cell types. In particular, it has been suggested that the activation of MAPKs by hypoxia or albumin is a function of ROS generation, but also angiotensin II and aldosterone modulate the activity of ERK1/2, JNK and p38 [16–19]. We thus sought to study the participation of ROS in the signal transduction pathway activated by DA in renal proximal tubule cells, and also to

Abbreviations: DA, dopamine; ERK1/2, extracellular signal-regulated kinases 1/2; MAO, monoaminoxidase; NF-κB, nuclear transcription factor κB; OK, opossum kidney cells; ROS, reactive oxygen species; SOD, superoxide dismutase

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investigate their possible role in the activation of MAPKs. We provide evidence of the generation of mitochondria-derived ROS even with low DA concentrations, suggestive of a messenger role for ROS in cellular redox regulation, and also of DA-induced activation of ERK1/2, nuclear transcription factor κB (NF-κB) and superoxide dismutase (SOD) at concentrations that exert a physiological regulation of renal function.

2. Materials and methods

2.1. Cell culture

OK cells, a cell line derived from Opossum kidney proximal tubular cells, were obtained from The American Type Culture Collection and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin/streptomycin (100 IU/ml and 100 μg/ml, respectively) and 2 mM L-glutamine in a humidified atmosphere of 5% CO_2/95% air at 37 °C, between passage 15 and 35.

2.2. Measurement of reactive oxygen species

ROS production was determined using 2′,7′-dichlorofluorescein diacetate (DCFH-DA). Cells were seeded, grown to 70% confluence, serum-starved for 24 h and then loaded for 30 min with DCFH-DA (10 μM) in serum-free DMEM at 37 °C. Thereafter, cells were washed twice with Hanks' Balanced Salt Solution (HBSS), incubated with the indicated agonists in HBSS and the fluorescence (485/20 and 528/20 nm excitation and emission respectively) assessed in a Biotek Instruments Synergy HT multiplate reader.

2.3. Western blot analysis

OK cells were seeded, grown to 70% confluence and serum-starved for 24 h before the experiments. After incubation with the different agonists, for MAPKs phosphorylation, cyclin D1, 5-LOX and COX-2 experiments, cells were scraped on ice in a buffer containing 20 mM Tris–HCl (pH 7.4), 0.5% Triton X-100, 1 mM EGTA, 1 mM EDTA, 150 mM NaCl, 50 mM NaF, 40 mM glycerophosphate, 1 mM PMSF, 2 mM sodium *ortho*vanadate, 1 μg/ml aprotinin, 10 μM leupeptin and 1 μM pepstatin. For translocation of Rel-A protein, nuclei were obtained by differential centrifugation as previously described [20]. Briefly, cells were washed in PBS, harvested and homogenized by mechanical disruption with a pellet pestle motor in a buffer containing 20 mM HEPES–KOH (pH 7.4), 0.25 M sucrose, 1 mM EDTA, protease and phosphatase inhibitors. Homogenates were centrifuged at 1000×*g* for 10 min and the resulting pellet was resuspended in the same buffer and nuclei disrupted by sonication.

Proteins were then separated on polyacrylamide gels, electrotransferred to PVDF membranes, incubated with antibodies against the phosphorylated forms of JNK (pJNK), p38 (pp38) and of ERK1/2 (pERK1/2) or against RelA (p65), cyclin D1, 5-lipooxigenase (5-LOX) and cyclooxygenase-2 (COX-2) and developed using the enhanced chemiluminescence reagent. Membranes were then stripped and reprobed using anti total ERK1/2, anti β-tubulin or anti histone H2B antibody as loading controls, and quantitated using the ImageQuant 5.2 software.

2.4. Superoxide dismutase activity determination

SOD activity was assayed as previously described [21]. Briefly, cells were serum-starved for 24 h, incubated with the different agonists, scraped on ice in a buffer containing 50 mM Tris (pH 7.4) and 1 mM EDTA, and sonicated for 30 s. The suspension was centrifuged at 12 000×*g* for 10 min at 4 °C and the resulting supernatant assayed in a reaction mixture containing 50 mM Tris (pH 8.6), 1 mM EDTA, 100 μM pyrogallol at 470 nm at 5 s intervals for 3 min in a Synergy HT multiplate reader against a blank containing all components except the enzyme preparation and pyrogallol. The rate of auto-oxidation of pyrogallol was considered as 100% auto-oxidation.

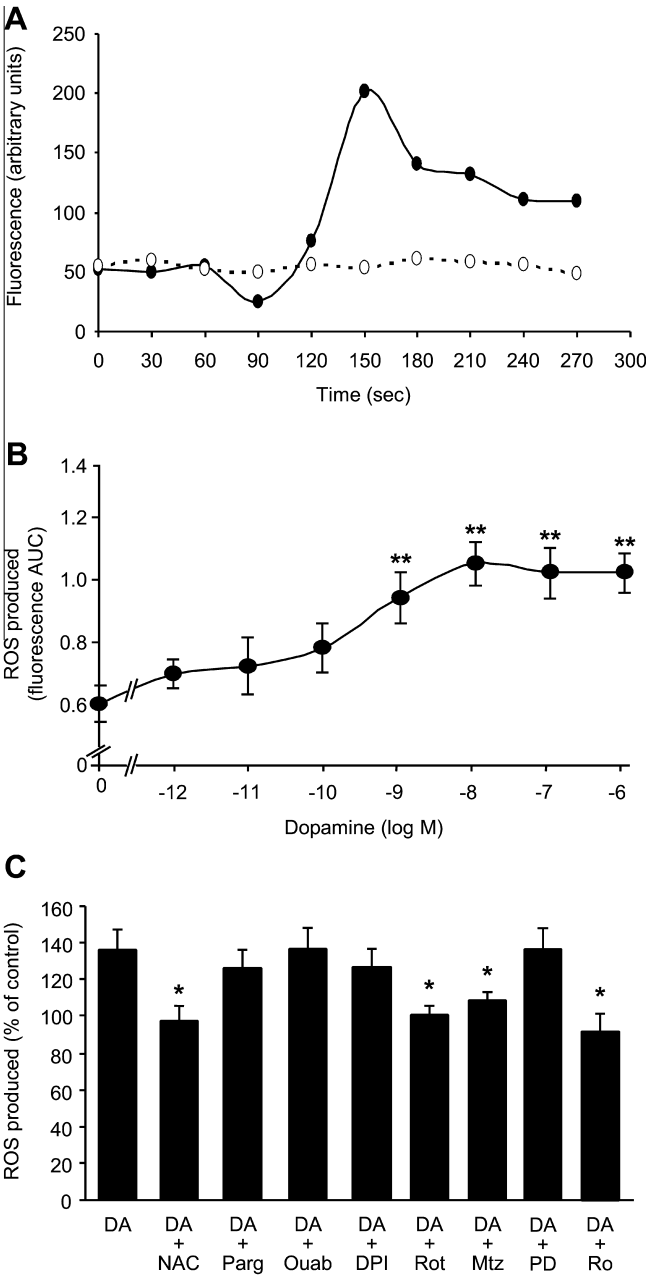

Fig. 1. Dopamine promotes ROS generation in OK cells. OK cells were serum-starved for 24 h, loaded with DCFH-DA and incubated with 1 μM DA (●) or its vehicle (○) for the indicated times (A), with the indicated concentrations of DA for 5 min (B) or with 1 μM DA alone or following preincubation (30 min) with 1 mM *N*-acetylcysteine (NAC), 1 μM pargyline (Parg), 100 μM ouabain (Ouab), 10 μM diphenyliodonium (DPI), 1 μM rotenone (Rot), 500 nM myxothiazol (Mtz), 50 μM PD98059 (PD) or 10 nM Ro 31-8220 (Ro) as indicated (C) and ROS levels measured by fluorescence. Results are expressed as fluorescence of a representative recording (A), integrated area under the curve (AUC) of the continuous 5 min reading (B) or as % of control of the AUC of a 5 min recording (C) and represent the mean ± S.E.M. arbitrary units of 5 experiments performed in triplicates. $^{**}P < 0.01$ vs. control (B); $^{*}P < 0.05$ vs. DA alone (C) by ANOVA followed by Tukey post test.

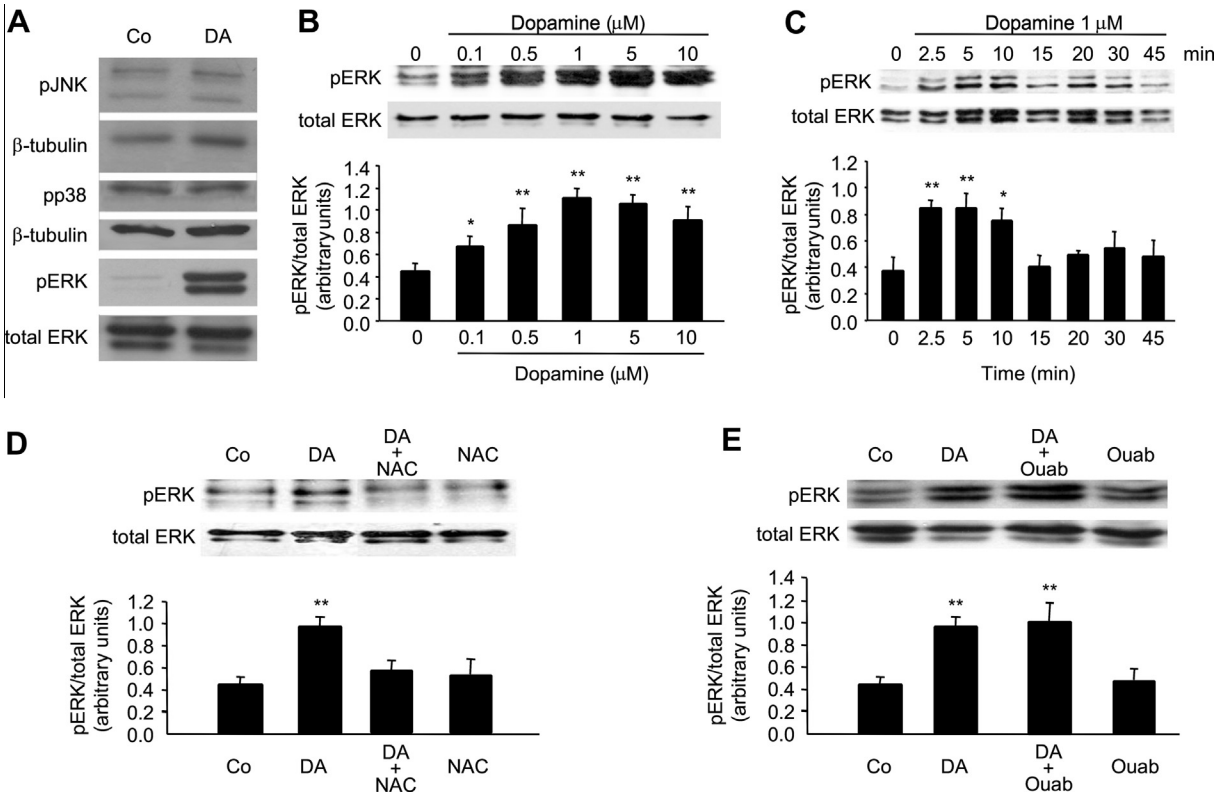

Fig. 2. Dopamine-induced ERK activation. OK cells were serum-starved for 24 h and incubated with 1 μM DA for 5 min (A, D and E) or during the indicated times (C) or with the indicated concentrations of DA for 5 min (B). Where indicated, cells were preincubated for 30 min with 1 mM *N*-acetylcysteine (NAC) or 100 μM ouabain (Ouab) prior to DA stimulation. Total cell lysates were immunoblotted using specific antibodies against pJNK, pp38 and pERK, and against β-tubulin or total ERK, and intensity of specific bands quantitated. Panel A shows representative immunoblots, whereas panels B–E show representative immunoblots and quantitations expressed as the mean ± S.E.M. of the ratio of phospho-ERK/total ERK in arbitrary units, in all cases of three independent experiments. $^{*}P < 0.05$; $^{**}P < 0.01$ vs respective control by ANOVA followed by Tukey test.

2.5. Catalase activity determination

Cells were serum-starved for 24 h, incubated with the different agonists, scraped on ice-cold PBS and centrifuged (1500×*g*, 4 °C) for 8 min. The pellet was suspended and lysed by repeated freezing and thawing in a buffer containing 50 mM potassium phosphate and 2% Triton X-100, pH 7.4. The extract was spun for 5 min at 1500×*g* and activity determined spectrophotometrically (240 nm) at 5 s intervals for 60 s after addition of H_2O_2 (36 v) to the supernatant as described [22].

2.6. Cell proliferation assay

Cells were serum-starved for 24 h and either incubated with 1 μM DA, vehicle or 10% FBS for 5 min, rinsed and cultured for 24, 48 or 72 h, or incubated with the same stimuli for 24, 48 or 72 h. In all cases, cell proliferation was determined at 24, 48 and 72 h after stimulation by staining with 0.05% crystal violet in 10% ethanol following fixation for 15 min in 10% formol. The dye was eluted with 50% distilled water, 50% ethanol and 0.1% glacial acetic acid and proliferation measured spectrophotometrically at 620 nm using a Synergy HT multiplate reader.

2.7. NF-κB activity determination

OK cells were seeded and grown up to 80% confluence, then transiently-transfected for 6 h with a eukaryotic expression vector carrying three consensus κB sequences fused in frame to the Firefly Luciferase-encoding cDNA (NF-κB-Luc), by lipofection in Opti-MEM medium and allowed to recover overnight in DMEM medium. Cells were then reseeded in 24-wells plates, grown for an extra 24 h period and serum-starved for 24 h prior to the experiment. Luciferase activity was measured using the Promega Luciferase Assay System in a Synergy HT multiplate reader and normalized using protein content of the respective sample.

3. Results

3.1. Dopamine stimulation of OK cells results in elevation of mitochondria-derived ROS

We first evaluated whether DA, in concentrations known to regulate sodium transport in proximal tubules, affects cellular redox state by on line recording of live DCFH-loaded OK cells. DA induced a rapid and time-dependent elevation of ROS intracellular levels (Fig. 1A). The effect of DA was also concentration-dependent, as DA produced a significant elevation of ROS when used at nanomolar concentrations and the effect was significant up to 10^{-6} M (Fig. 1B).

Cells were pretreated with pargyline, diphenyliodonium, rotenone, myxothiazol, Ro 31–8220, PD98059 and ouabain as MAO, NADPH oxidase, mitochondrial complexes I and III, PKC, ERK1/2 activating pathway and Na^+, K^+-ATPase inhibitors, respectively to determine the source of ROS. Neither pargyline, nor DPI, PD98059 or ouabain changed the effect of DA, whereas rotenone, myothiazol and Ro 31–8220 abolished the DA-mediated rise in ROS. Expectedly, the antioxidant *N*-acetyl-L-cysteine (NAC) blunted DA-mediated ROS generation (Fig. 1C).

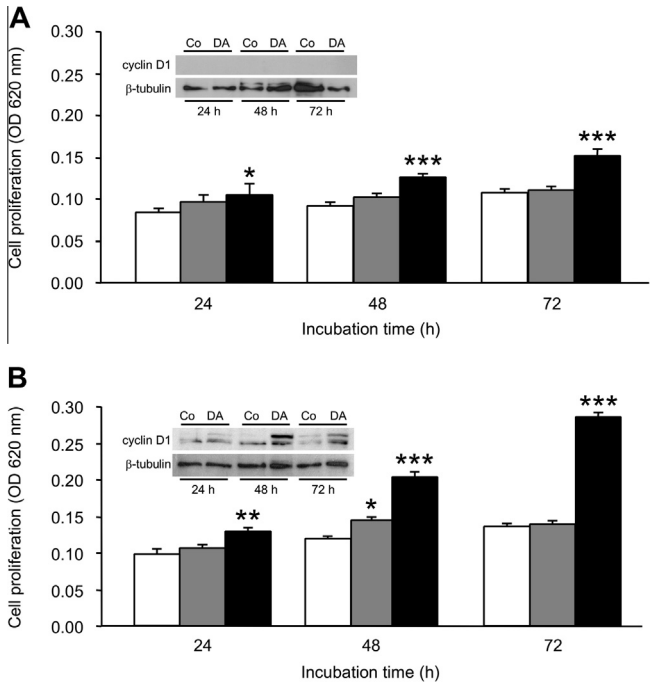

Fig. 3. Effect of dopamine on OK cell proliferation. OK cells were serum-starved for 24 h and incubated with 1 μM DA (■), 10% fetal bovine serum (■) or vehicle (□) for 5 min and proliferation evaluated after 24, 48 or 72 h culture in fresh serum-free medium (wash-out protocol, A) or with 1 μM DA (■), 10% FBS (■) or vehicle (□) for 24, 48 or 72 h prior to the determination of cell proliferation (continuous exposure protocol, B) by crystal violet staining. Insets show the effect of 1 μM DA or its vehicle (Co) on cyclin D1 expression in OK cell homogenates for the wash-out protocol (A) or continuous exposure protocol (B) by Western blot. For crystal violet staining, results are expressed as the mean ± S.E.M. OD 620 nm, whereas for Western blots the figure shows representative chemiluminescence detections of cyclin D1 and β-tubulin (loading control) by specific antibodies. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ vs respective control by ANOVA followed by Tukey test.

3.2. Dopamine promotes the phosphorylation of ERK1/2

Next, the effect of DA on MAPKs phosphorylation was examined. DA promoted the phosphorylation of ERK1/2, whereas both JNK and p38 were unaffected (Fig. 2A). DA promoted a concentration-, and time-dependent activation of ERK1/2 as the effect was evident when cells were exposed to 10^{-7} M and maximum at 10^{-6} M DA (Fig. 2B) and it showed a rapid onset, 2.5 min, peaked at 5 min and ceased after 15 min of stimulation (Fig. 2C). NAC blunted DA-mediated ERK1/2 activation, whereas ouabain (10^{-4} M) had no effect on either basal or DA-activated ERK1/2 phosphorylation (Fig. 2D and E).

In view of the effect of DA on ERK activation, we tested if DA increased cell proliferation. For that purpose, serum-starved cells were incubated in the presence of 10^{-6} M DA for 5 min, after which the medium was replaced and cells allowed to grow for up to 72 h after stimulation, or the cells were incubated in the presence of DA for 24, 48 or 72 h. A 5 min DA pulse affected neither cell proliferation nor cyclin D1 expression, which remained undetected under these conditions (Fig. 3A). A significant effect of DA was observed only when cells were continuously exposed to DA for 48 h (Fig. 3B), an effect that is also manifested by an increase in cyclin D1 levels while, expectedly, addition of 10% fetal bovine serum to the culture produced a marked stimulation of cell proliferation.

3.3. Dopamine mediates the stimulation of SOD but not of CAT activity

We also investigated if DA-mediated effect on ROS levels impacted on SOD and CAT activity. DA (10^{-6} M) increased SOD activity in a time-, and concentration-dependent fashion (Fig. 4A and B) and the effect was blunted by NAC, Ro 31–8220 and PD98059 (Fig. 4C). On the other hand, DA had no effect on CAT activity at the concentrations and times employed in this study (Fig. 4D).

3.4. Dopamine activates NF-κB

The effect of DA on NF-κB on live OK cells transiently-transfected with a vector carrying three consensus κB sequences fused to Firefly Luciferase-encoding cDNA (NF-κB-Luc) was also studied. DA (10^{-6} M) promoted a time-dependent activation of NF-κB (Fig. 5A). The activation of NF-κB was accompanied by translocation of RelA (p65) to the nucleus, as indicated by Western blot analysis of nuclear extracts of DA-treated OK cells (Fig. 5B). Expectedly, the effect of DA was abolished by preincubation of the cells with NAC and parthenolide (an inhibitor of NF-κB activity). NF-κB activation was dependent on PKC and on ERK1/2 activation, as observed when cells were preincubated in the presence of Ro 31–8220 and PD90859, respectively (Fig. 5B).

3.5. Dopamine induces the expression of 5-lipoxygenase and of cyclooxygenase-2

5-LOX and COX-2 are known targets of NF-κB regulation. Thus, we have also explored the effect of DA on the expression of both enzymes in OK cells. Incubation of the cells with 10^{-6} M DA resulted in a time-dependent increase in protein expression of both 5-LOX and COX-2, an effect that became evident only after 30 min of incubation and resulted maximal after 120 min of DA exposure (Fig. 6).

4. Discussion

The present study demonstrates that dopamine influences the redox state of OK cells via the generation of mitochondrial ROS. We have used DCFH [23] to analyze the effect of DA on ROS levels and found that DA promotes a fast and concentration-dependent elevation of ROS, within the time frame and at concentrations that exert a physiological regulation of renal function.

Reactive oxygen species are produced in all aerobic organisms as a result of metabolic processes or due to noxious agents. Although the reactivity of ROS explains the deleterious effects they can cause, low levels are generated in a controlled manner to act as regulators of redox-sensitive processes. Indeed, increasing evidence supports a signaling role for ROS in the regulation of cell growth, proliferation, migration and apoptosis [24].

In mammalian cells, ROS are mainly produced by the mitochondria and by NADPH-oxidase. Our experiments implicated mitochondria as the major source of ROS. The specific site(s) within the mitochondria where ROS are produced is a matter of debate, but complexes I and III are often implicated [25]. Both rotenone and myxothiazol substantially reduced DA-induced ROS, indicating that they are produced at both complexes in OK mitochondria. Moreover, we can rule out MAO degradation and Na^+,K^+-ATPase inhibition as possible sources of ROS in OK cells, as pargyline and ouabain did not modify the effect of DA. MAO could contribute to ROS generation since it produces H_2O_2 during DA metabolism and ROS elevations secondary to Na^+,K^+-ATPase inhibition have been reported in cardiac myocites [26]. The fact that DA effect was blunted in the presence of a PKC inhibitor excludes also a non-specific effect and suggests the involvement of PKC in the process.

The participation of MAPKs in DA-activated signal cascade was also explored. Of the kinases assayed, ERK1/2 became phosphory-

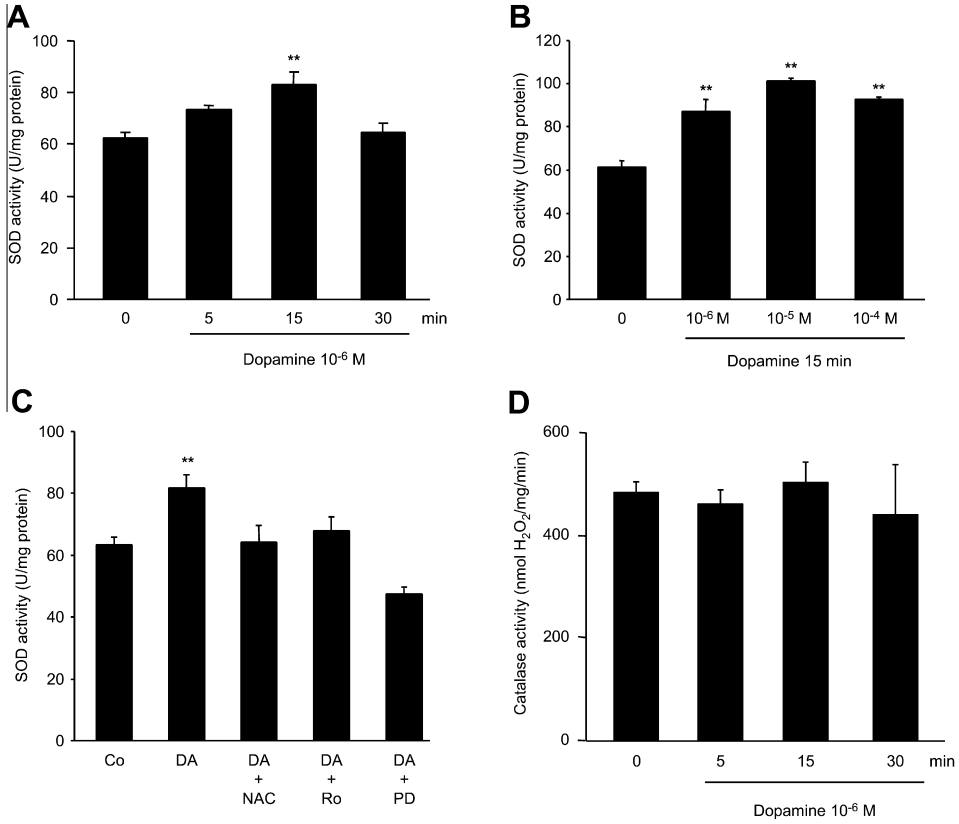

Fig. 4. Effect of dopamine on SOD and CAT activity. OK cells were serum-starved for 24 h and incubated with 1 μM DA for the indicated times (A and D), with the indicated DA concentrations during 15 min (B) or with 1 μM DA or vehicle (Co) for 15 min following preincubation of the cells (30 min) with 1 mM *N*-acetylcysteine (NAC), 10 nM Ro 31-8220 (Ro) or 50 μM PD98059 (PD) as indicated (C) and SOD (A–C) or CAT (D) determined. Results are expressed as the mean ± S.E.M. U/mg protein or nmol H_2O_2/mg protein/min for SOD and CAT, respectively. $^{**}P < 0.01$ vs respective control by ANOVA followed by Tukey test.

lated (thus activated) under the same conditions that elevate ROS levels. Moreover, our results indicate that ERK1/2 become activated in response to ROS elevations, thus pointing to a downstream function in the signal transduction cascade. Concordant with that observed for ROS, ouabain had no effect on ERK1/2 phosphorylation.

When present at high and/or sustained levels ROS can cause severe damage thus, cells carry an intricate antioxidant defense system including enzymatic and non-enzymatic scavengers. Our experiments show a rapid and transient increase of SOD, with no effect on CAT activity, on cells exposed to DA. Although an activation of antioxidant defenses seems logical following ROS generation, the current paradigm of SOD regulation is based on transcriptional control [27]. One possible explanation is a rapid posttranlational regulation of SOD activity, to prevent damage from O_2^-. One such mechanism has been described in bacteria [28]. Our experiments show that DA-induced SOD activation is prevented by inhibition of the ERK1/2 pathway.

Since ERK signaling is involved in cell proliferation, we tested whether DA promotes cell growth. Under the conditions employed here DA, at concentrations and times that result in ROS generation and ERK1/2 activation, produced no effect on cell proliferation. Only after continuous exposure to DA for 48 h does cell proliferation become significantly elevated over controls. Similar results were obtained by studying cyclin D1 expression in OK cell lysates by Western blot. Cyclin D1 was detected in the homogenates of cells that were exposed to DA for at least 24 h. In cells subjected to the wash-out protocol, cyclin D1 was below detection levels for the different conditions studied and became apparent only after prolonged overexposure of the immunoblots. Thus, our results suggest that activation of ERK1/2, in the context of DA regulation of proximal tubule cells, may serve to regulate transport mechanisms and not to control cell proliferation. In view of our results linking ERK and SOD activity, a role for ERK1/2 in the regulation of the antioxidant response seems plausible.

In our experiments, DA also increased the activity of a reporter plasmid carrying a NF-κB binding consensus sequence. The effect of DA was time-dependent with a significant increase in luciferase activity occurring as early as 5 min. The involvement of NF-κB in DA signaling pathway was further evidenced by the nuclear translocation of RelA (p65) as shown in Fig. 5B. The effect of DA on NF-κB was blunted by preincubation of the cells with a ROS scavenger and with the NF-κB inhibitor parthenolide. Therefore, we conclude that NF-κB activation is an event dependent on ROS. In addition, the fact that Ro 31–8220 and PD98059 abolish NF-κB activation suggest that PKC and ERK1/2 are involved in this activation.

A myriad of stimuli can rapidly activate the Rel/NF-κB family of eukaryotic transcription factors by freeing them from their inhibitor and enabling them to translocate to the nucleus. Active NF-κB promotes, in its turn, the expression of a diverse array of target genes [29]. Products of the cyclooxygenases, 5-lipoxygenase, and cytochrome P450 pathways of arachidonic acid metabolism play a major role in renal physiology and hemodynamics. In view of that, we have explored a possible effect of DA on two well recognized targets of NF-κB, namely 5-LOX and COX-2. We found that DA regulates the expression of both 5-LOX and of COX-2 in OK cells. Together, these results indicate that ROS-induced ERK1/2 activation that follows DA stimulation would also result in NF-κB activation and, consequently, in the regulation of eicosanoid metabolism possibly through NF-κB-dependent gene transcription.

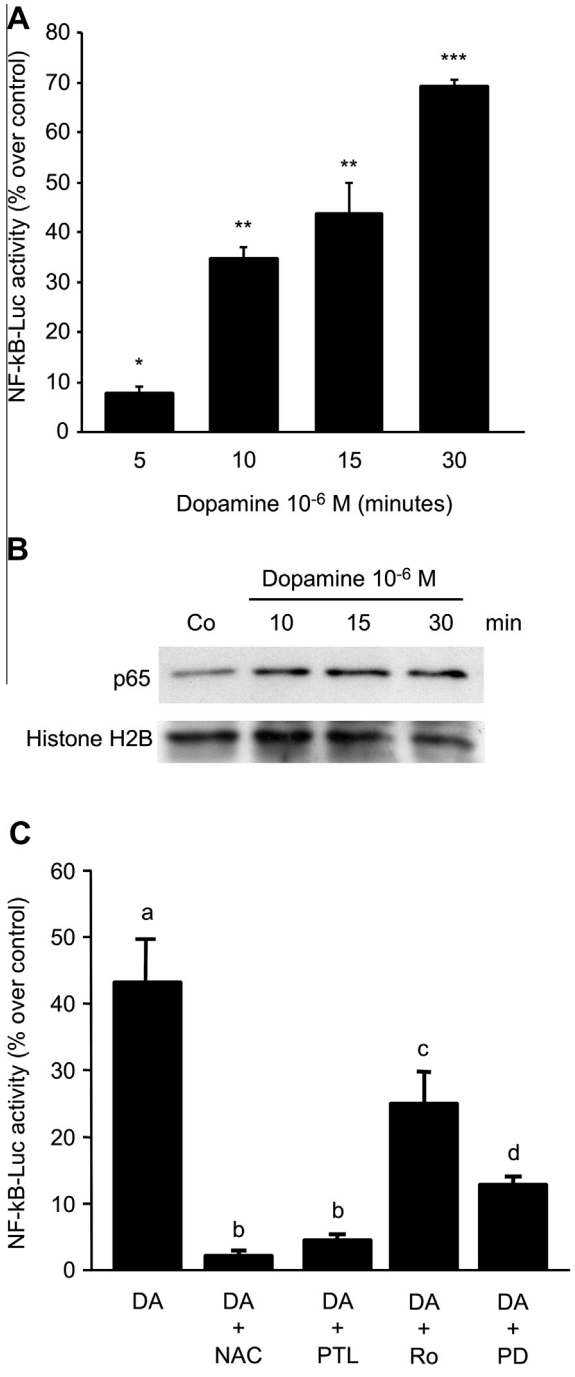

Fig. 5. Effect of dopamine on NF-κB activity. OK cells were transiently-transfected with a eukaryotic expression vector carrying an NF-κB-Luc encoding cDNA, grown for an extra 24 h period and serum-starved for 24 h prior to incubation with 1 μM DA for the indicated times (A) or for 15 min, alone or following a 30 min preincubation with 1 mM *N*-acetylcysteine (NAC), 5 μM parthenolide (Ptl), 10 nM Ro 31-8220 (Ro) or 50 μM PD98059 (PD) as indicated (C) and luciferase activity determined, or serum-starved for 24 h and incubated with 1 μM DA for the indicated times, homogenized and nuclei separated by subcellular fractionation and nuclear proteins subjected to immunoblot using specific anti Rel-A (p65) and Histone H2B (loading control) antibodies (B). Results represent NF-κB-Luc activity and are expressed as the mean ± S.E.M. % of control of three independent experiments performed in triplicates (A and C) or a representative immunoblot performed as described in Section 2 (B). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ vs control by ANOVA followed by Tukey test. (a) $P < 0.01$ vs control; (b) $P < 0.001$; (c) $P < 0.05$; (d) $P < 0.01$ vs dopamine, by Student's *t*-test.

Our findings offer new insights into the mechanism of action of dopamine within the renal proximal tubule by confirming the participation of ROS in its signal transduction cascade. An integrated

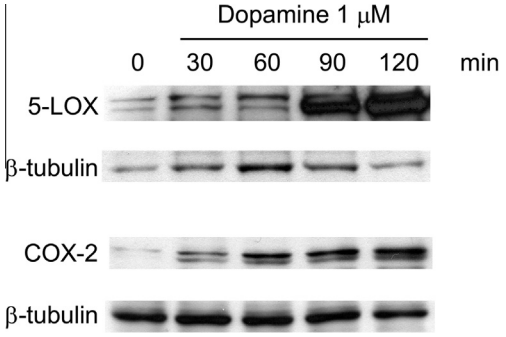

Fig. 6. Effect of dopamine on 5-lipooxigenase (5-LOX) and on ciclooxigenase-2 (COX-2) expression. OK cells were serum-starved for 24 h and incubated with 1 μM DA for the indicated times. Total cell lysates were immunoblotted using specific antibodies against 5-LOX and COX-2, and against β-tubulin as loading control. The figure show representative immunoblots of experiments performed in triplicates.

view of these findings implies that DA activates PKC and promotes ROS generation at the mitochondria. Generated ROS will, in turn, activate ERK1/2 that will stimulate SOD activity and also NF-κB. ROS generation in the context of the physiological regulation of water and electrolyte transport is indicative of a redox-based control mechanism and our results are therefore consistent with a physiological setup in which they are part of DA's signal transduction pathway. Thus, we interpret our data as evidence of a messenger role for ROS in OK cells, a role that implies also an adaptative effect that could prevent damage through a rapid up-regulation of antioxidant defenses and by a later transcriptional regulation if cells are exposed to DA in either high concentrations or for longer periods of time.

Acknowledgments

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References

[1] Bertorello, A., Hökfelt, T., Goldstein, M. and Aperia, A. (1988) Proximal tubule Na+–K+-ATPase activity is inhibited during high-salt diet: evidence for DA-mediated effect. Am. J. Physiol. 254, F795–F801.
[2] Finkel, Y., Eklöf, A.C., Granquist, L., Soares-da-Silva, P. and Bertorello, A.M. (1994) Endogenous dopamine modulates jejunal sodium transport during high salt diet in young but not in adult rats. Gastroenterology 107, 675–679.
[3] Tayebati, S.K., Lokhandwala, M.F. and Amenta, F. (2011) Dopamine and vascular dynamics control: present status and future perspectives. Curr. Neurovasc. Res. 8, 246–257.
[4] Barnard, M.L., Olivera, W.G., Rutschman, D.M., Bertorello, A.M., Katz, A.I. and Sznajder, J.I. (1997) Dopamine stimulates sodium transport and liquid clearance in rat lung epithelium. Am. J. Respir. Crit. Care Med. 156, 709–714.
[5] Bindoli, A., Rigobello, M.P. and Deeble, D.J. (1992) Biochemical and toxicological properties of the oxidation products of catecholamines. Free Radic. Biol. Med. 13, 391–405.
[6] Brunmark, A. and Cadenas, E. (1988) Oxidation of quinones by H_2O_2: formation of epoxy- and hydroxyquinone adducts and electronically excited states. Basic Life Sci. 49, 81–86.
[7] Michel, P. and Hefti, F. (1990) Toxicity of 6-hydroxydopamine and dopamine for dopaminergic neurons in culture. J. Neurosci. Res. 26, 428–435.
[8] Hastings, T.G., Lewis, D.A. and Zigmond, M.J. (1996) Role of oxidation in the neurotoxic effects of intrastriatal dopamine injections. Proc. Natl. Acad. Sci. USA 93, 1956–1961.

[9] Berman, S. and Hastings, T. (1997) Inhibition of glutamate transport in synaptosomes by dopamine oxidation and reactive oxygen species. J. Neurochem. 69, 1185–1195.
[10] Haque, M.E., Asanuma, M., Higashi, Y., Miyazaki, I., Tanaka, K. and Ogawa, N. (2003) Apoptosis-inducing neurotoxicity of dopamine and its metabolites via reactive quinone generation in neuroblastoma cells. Biochim. Biophys. Acta 1619, 39–52.
[11] Berman, S. and Hastings, T. (1999) Dopamine oxidation alters mitochondrial respiration and induces permeability transition in brain mitochondria: implications for Parkinson's disease. J. Neurochem. 73, 1127–1137.
[12] Asanuma, M., Miyazaki, I. and Ogawa, N. (2003) Dopamine- or L-DOPA-induced neurotoxicity: the role of dopamine quinine formation and tyrosinase in a model of Parkinson's disease. Neurotox. Res. 5, 165–176.
[13] Paravicini, T.M. and Touyz, R.M. (2006) Redox signaling in hypertension. Cardiovasc. Res. 71, 247–258.
[14] Zeng, C., Villar, V.A., Yu, P., Zhou, L. and Jose, P.A. (2009) Reactive oxygen species and dopamine receptor function in essential hypertension. Clin. Exp. Hypertens. 31, 156–178.
[15] Schafer, F.Q. and Buettner, G.R. (2001) Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic. Biol. Med. 30, 1191–1212.
[16] Aoto, M., Shinzawa, K., Suzuki, Y., Ohkubo, N., Mitsuda, N. and Tsujimoto, Y. (2009) Essential role of p38 MAPK in caspase-independent, iPLA(2)-dependent cell death under hypoxia/low glucose conditions. FEBS Lett. 583, 1611–1618.
[17] Reich, H., Tritchler, D., Herzenberg, A.M., Kassiri, Z., Zhou, X., Gao, W. and Scholey, J.W. (2005) Albumin activates ERK via EGF receptor in human renal epithelial cells. J. Am. Soc. Nephrol. 16, 1266–1278.
[18] Harvey, B.J., Alzamora, R., Stubbs, A.K., Irnaten, M., McEneaney, V. and Thomas, W. (2008) Rapid responses to aldosterone in the kidney and colon. J. Steroid Biochem. Mol. Biol. 108, 310–317.
[19] Alexander, L.D., Ding, Y., Alagarsamy, S., Cui, X.L. and Douglas, J.G. (2006) Arachidonic acid induces ERK activation via Src SH2 domain association with the epidermal growth factor receptor. Kidney Int. 69, 1823–1832.
[20] Brion, L., Maloberti, P.M., Gomez, N.V., Poderoso, C., Gorostizaga, A.B., Mori Sequeiros Garcia, M.M., Acquier, A.B., Cooke, M., Mendez, C.F., Podesta, E.J. and Paz, C. (2011) MAPK phosphatase-1 (MKP-1) expression is up-regulated by hCG/cAMP and modulates steroidogenesis in MA-10 Leydig cells. Endocrinology 152, 2665–2677.
[21] Marklund, S.L. and Marklund, G. (1974) Involvement of superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem. 47, 469–474.
[22] Claiborne, A. (1985) Catalase activity in: Handbook of Methods for Oxygen Radical Research (Greewald, R.A., Ed.), pp. 283–284, CRC Press, Boca Raton.
[23] Kalyanaraman, B., Darley-Usmar, V., Davies, K.J., Dennery, P.A., Forman, H.J., Grisham, M.B., Mann, G.E., Moore, K., Roberts 2nd, L.J. and Ischiropoulos, H. (2012) Measuring reactive oxygen and nitrogen species with fluorescent probes: challenges and limitations. Free Radic. Biol. Med. 52, 1–6.
[24] Bartosz, G. (2009) Reactive oxygen species: destroyers or messengers? Biochem. Pharmacol. 77, 1303–1315.
[25] Chandel, N.S. and Schumacker, P.T. (2000) Cellular oxygen sensing by mitochondria: old questions, new insight. J. Appl. Physiol. 88, 1880–1889.
[26] Tian, J., Gong, X. and Xie, Z. (2001) Signal-transducing function of Na^+–K^+-ATPase is essential for ouabain's effect on $[Ca^{2+}]_i$ in rat cardiac myocytes. Am. J. Physiol. Heart Circ. Physiol. 281, H1899–H1907.
[27] Zelko, I., Mariani, T. and Folz, R. (2002) Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution and expression. Free Radic. Biol. Med. 33, 337–349.
[28] Archambaud, C., Nahori, M.A., Pizarro-Cerda, J., Cossart, P. and Dussurget, O. (2006) Control of *Listeria* superoxide dismutase by phosphorylation. J. Biol. Chem. 281, 31812–31822.
[29] Pahl, H. (1999) Activators and target genes of Rel/NF-kB transcription factors. Oncogene 18, 6853–6866.