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Dopamine D_2-like receptor agonist bromocriptine protects against ischemia/reperfusion injury in rat kidney

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Dopamine D_2-like receptor agonist bromocriptine protects against ischemia/reperfusion injury in rat kidney.

Background. Dopamine, via activation of D_1-like and D_2-like receptors, plays an important role in the regulation of renal sodium excretion. Recently, we demonstrated that dopamine D_2-like receptor agonist (bromocriptine) stimulates p44/42 mitogen-activated protein kinases (MAPKs) and Na^+,K^+ATPase (NKA) activity in proximal tubular epithelial cells. Since both these parameters are compromised in ischemia/reperfusion (I/R) injury to the kidney, we investigated whether bromocriptine protects against the injury.

Methods. In this study we used unilateral rat model of renal I/R injury. The Sprague-Dawley rats were divided into vehicle and bromocriptine groups. The vehicle and bromocriptine group was treated with vehicle and bromocriptine (500 μg/kg intravenously), respectively, 15 minutes before the induction of unilateral ischemia followed by 24- or 48-hour reperfusion. At the end of 24 or 48 hours the animals were sacrificed to collect control and ischemic kidney cortices, in which necrosis, apoptosis, NKA activity, NKA $\alpha 1$ subunit expression, and p44/42 MAPK phosphorylation were measured.

Results. We found extensive necrosis, apoptosis, and decreased NKA activity (with no change in $\alpha 1$ subunit) in the ischemic kidney cortex compared to the nonischemic cortex from the vehicle-treated rats as early as 24 hours post-reperfusion. In contrast, I/R injury–induced necrotic, apoptotic, and decrease in NKA activity were absent in the outer cortex of bromocriptine-treated rats after 24 or 48 hours. Interestingly, we detected significantly higher phosphorylation of p44/42 MAPKs in control and ischemic kidneys of bromocriptine-treated rats compared to those of vehicle-treated rats.

Conclusion. Therefore, bromocriptine, a D_2-like receptor agonist, may protect against I/R injury to proximal tubules of the kidney, via p44/42 MAPK activation.

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Ischemia/reperfusion (I/R) injury in kidney is one of the common causes of acute renal failure. Renal I/R injury is usually associated with decreases in renal blood flow (RBF) and glomerular filtration rate (GFR), an increase in fractional excretion of sodium, and increased serum creatinine and blood urea nitrogen (BUN) levels [1–5]. These functional defects in the ischemic kidney are in part caused by necrosis and apoptosis of the tubular epithelial cells during I/R injury [6–8]. It has been suggested that proximal tubules and medullary thick ascending limb sustain maximum damage during I/R injury, with proximal tubules being most susceptible of the two [9–11].

Multiple cellular factors are involved in the post-I/R injury events, which lead to acute renal failure [12–14]. Recently, several groups have begun to explore the role of mitogen-activated protein kinases (MAPKs) in I/R injury [4, 5, 10, 15–17]. MAPKs are a class of protein kinases, which play an important role in the determination of cell survival. While p44/42 MAPKs attenuate apoptosis, p38 and JNK promote apoptosis [18]. The differential regulation of these three MAPKs is reported in I/R injury to cardiac myocytes [16]. Further, Di Mari et al demonstrated that differential regulation of p44/42, p38 and JNK MAPKs determines the fate of the tubular epithelial cells in renal I/R injury [10]. More specifically, selective activation of JNK, but not of p44/42 MAPK, leads to extensive apoptosis observed in the proximal tubules after I/R injury [10, 17]. Therefore, drugs that activate p44/42 MAPKs and/or inactivate JNK may protect the kidney, especially the proximal tubules, against I/R injury.

Dopamine plays an important role in the regulation of renal function [19, 20]. Moreover, the in vivo and in vitro pharmacologic effects of dopamine receptor agonists in the kidney have been extensively studied [19, 20]. We recently showed that bromocriptine (D_2-like receptor

agonist) stimulates p44/42 MAPKs in the proximal tubules of the kidney [21, 22], which is a potential target to counter I/R injury. Since proximal tubules are maximally affected in the course of renal I/R injury [9–11], we hypothesized that bromocriptine may protect against I/R injury to the proximal tubules of the kidney.

The proposed hypothesis was tested using unilateral rat model of renal I/R injury. In this model, one kidney serves as a sham control and the other kidney is subjected to 45 minutes of ischemia followed by reperfusion. Using the unilateral rat model, we previously showed that the kidney subjected to I/R injury undergoes extensive necrosis and apoptosis compared to sham operated kidney within 24 hours after reperfusion [17, 23]. Therefore, in studying the reno-protective effects of bromocriptine, we evaluated necrosis and apoptosis in rat kidney as biomarkers of I/R injury. Moreover, in terms of sodium handling, I/R injury induces the loss of major sodium transporters in the kidney and leads to an increase in fractional sodium excretion [2]. Interestingly, bromocriptine increases the activity of at least one such transporter Na^+, K^+, ATPase (NKA) via p44/42 MAPK pathway in renal proximal tubules [22]. On the basis of this observation, we additionally measured the effect of bromocriptine on NKA activity and protein expression in I/R injury.

The experiments were performed in Sprague-Dawley rats treated with vehicle or bromocriptine before induction of I/R injury. In both groups, the above three parameters were measured in the outer renal cortical regions (rich in proximal tubules) of sham control and I/R injury kidney. Furthermore, p44/42 phosphorylation (activation) was also measured in both groups to examine whether its induction coincided with the protective effects of bromocriptine in I/R injury.

METHODS

All animal experiments were carried out according to the principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication 86–23, revised 1989, authorization 00577, 1989, Paris, France). Male Sprague-Dawley rats weighing 140 to 190 g (Harlan ZI Du Malcourlet, France) were housed individually in standard laboratory cages with ad libitum access to food and water.

Unilateral renal I/R injury in Sprague-Dawley rats

In this procedure, the rats were anesthetized with sodium pentobarbital (60 mg/kg intraperitoneally). Next, the jugular vein was cannulated with a polyethylene catheter (PE-10) for the administration of the drug. Ischemia was induced by clamping the right renal artery with nontraumatic vascular clip for 45 minutes. During

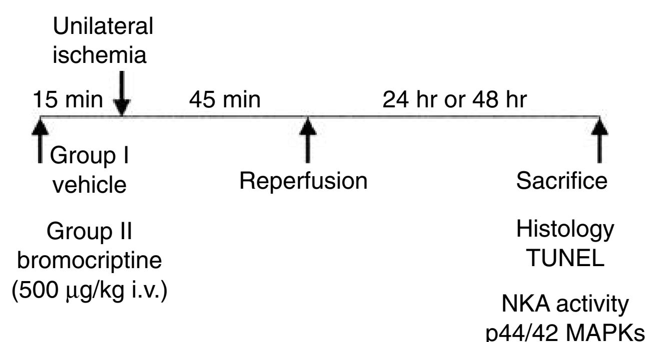


Fig. 1. Schematic representation of induction of renal ischemic/reperfusion (I/R) injury.

the operation, the animals were kept on a surgical thermostatically controlled table at $38 \pm 1°C$ under anesthesia. After surgery the animals were returned to the cages, where they had free access to food and water. The rats were divided into two sets (24-hour and 48-hour sets), with each set having two groups. In each set, one group of animals ($N = 6$) was given bromocriptine (500 μg/kg intravenously) 15 minutes prior to the renal artery occlusion, whereas a second group ($N = 6$) received vehicle. Experimental protocol is explained in Figure 1. The contralateral nonischemic kidney serves as a control to the ischemic kidney. The kidneys from 24- or 48-hour set of rats were removed after 24 hours or 48 hours of reperfusion, respectively, and divided lengthways into two parts. One part was suspended in ice-cold modified Krebs-Henseleit buffer (KHB) solution [22] for biochemical assays and the second was fixed in Dubose solution for histologic examination.

Histologic examination

For histologic examination, Dubose-fixed and paraffin-embedded kidney specimens (5 μm) were stained with hematoxylin and eosin. Histologic changes were evaluated by measurement of tissue necrosis graded on an A to F scale in relation to the extent of kidney damage: A, none; B, up to 10%; C, from 10% to 25%; D, from 25% to 50%; E, from 50% to 75%; and F, more than 75%. Tubular necrosis was assessed in the renal cortex at 24 hours and 48 hours after I/R.

TUNEL assay

The sections of kidney embedded in paraffin were deparaffinized in toluene and a graded series of ethanol. DNA fragmentation (apoptosis) was visualized in situ on fixed tissues by the terminal transferase-mediated deoxyuridine triphosphate (dUTP) nick-end labeling (TUNEL) procedure, using the apoptosis detection kit of Promega (Madison, WI, USA). Briefly, the deparaffinized sections were incubated in proteinase K

(20 μg/mL) solution to permeabilize the tissues, rinsed, and further fixed in paraformaldehyde (4%). The sections were incubated with 1 μL of terminal deoxynucleotidyl transferase (25 U/μL) and fluorescein-12-dUTP in equilibration buffer [25 mmol/L Tris-HCl, pH 6.6; 200 mmol/L potassium cacodylate, pH 6.6, 2.5 mmol/L cobalt chloride, 0.25 mg/mL bovine serum albumin (BSA), and 0.2 mmol/L dithiothreitol (DTT)] for 1 hour at 37°C. Further, the slides were rinsed four times in standard sodium citrate (SSC) and phosphate-buffered saline (PBS) and immersed in 40 mL propidium iodide solution (1 μg/μL) for 15 minutes. The positive control was treated by DNAse I (1 μg/μL) before being processed with the TUNEL procedure. The TUNEL-positive cells were counted under fluorescence microscope and data represented as percent of cells that stained TUNEL positive.

Preparation of cortical homogenate

The kidneys isolated from the above groups were immediately transferred to 5 mL ice-cold KHB solution containing protease inhibitor cocktail. Outer cortex was separated from each kidney, to obtain the portion rich in proximal tubules. The isolated cortical slices were homogenized using Wheaton's homogenizer at setting 7 for 20 strokes, and the protein concentration of the homogenate measured. The NKA activity, NKA α1-subunit expression, and p44/42 MAPKs phosphorylation were measured in the cortical homogenate.

NKA activity in cortical homogenate

The NKA activity was measured in cortical homogenate (1 mg/mL) as previously described [28]. The reaction mixture was carried out in 1.025 mL of assay buffer [37.5 mmol/L immidazole buffer, 70 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L Na ethylenediaminetetraacetic acid (EDTA), 5 mmol/L $MgCl_2$, 6 mmol/L NaN_3, 75 mmol/L Tris-HCl] using 0.1 mL (0.1 mg protein) of cortical homogenate. The reaction was initiated by adding 4 mmol/L adenosine triphosphate (ATP). Ouabain-insensitive ATPase activity was determined in parallel using an assay buffer containing 150 mmol/L Tris-HCl, 1 mmol/L ouabain instead of NaCl and KCl. The reaction was carried out at 37°C for 15 minutes and terminated by adding 0.05 mL of ice-cold 50% trichloroacetic acid (TCA). The NKA activity was measured as the function of liberated inorganic phosphate (Pi) in triplicates. The NKA activity was calculated as the difference between the total and ouabain-insensitive ATPase activity and was represented as Pi μmol/mg protein/min.

NKA α1 subunits and p44/42 MAPKs in cortical homogenate by Western blotting

Loading samples were prepared for western blotting containing sodium dodecyl sulfate (SDS)-Laemmli (4×) (0.05 mL), bromophenol blue (0.02 mL), corticular homogenate (0.1 mL), and water (0.03 mL). Further, the loading samples were resolved by SDS-polyacrylamide gel electrophoresis (PAGE) and transferred to polyvinylidene fluoride (PVDF) membrane. The NKA α1 subunits, phospho-p44/42 MAPKs, and total MAPKs on the PVDF membrane were detected using monoclonal NKA α1-subunit antibody (Research Diagnostics, Flanders, NJ, USA), anti-phospho-p44/42 MAPKs antibody (Promega), and anti-p42 MAPKs antibody (Santa Cruz Biotechonology, Santa Cruz, CA, USA), respectively. Where indicated, the Western blots were subjected to densitometric analysis using Scion Image software. The MAPKs data are represented as the densitometric ratio of phospho- to total-p42 MAPKs.

Table 1. Histologic changes

Grading scale	Tubular necrosis %	Vehicle + I/R		Bromocriptine + I/R	
		24 hours	*48 hours*	*24 hours*	*48 hours*
A	0	–	–	4	3
B	0–10	–	–	1	2
C	10–25	1	–	–	–
D	25–50	1	1	–	–
E	50–75	2	2	–	–
F	>75	1	2	–	–

I/R is ischemic/reperfusion injury. Histologic changes were evaluated by measuring of tissue necrosis graded on a 0 to 5 scale in relation to the extent of kidney damage: A, none; B, up to 10%; C, from 10% to 25%; D, from 25% to 50%; E, from 50% to 75%; and F, more than 75%. The grading was performed in cortical regions of ischemic kidneys from vehicle-treated and bromocriptine-treated rats.

Data analysis

Where applicable, data are presented as mean ± SEM of number (N) of experiments. Statistical analysis was done using unpaired Student t test or one-way analysis of variance (ANOVA) as explained in the figure legends. The difference was considered statistically significant at $P < 0.05$.

RESULTS

Necrosis in the cortical slices of vehicle- and bromocriptine-treated rats

The histologic alterations induced by unilateral renal ischemia were evaluated at 24 hours and 48 hours of reperfusion. Extensive necrosis was observed in outer cortex of ischemic compared to contralateral kidneys of vehicle-treated rats after 24 hours of reperfusion (Fig. 2A and B, Table 1) and 48 hours of reperfusion (Table 1). On the other hand, the histology of ischemic cortex from bromocriptine (500 μg/kg intravenously)-treated rats was similar to contralateral kidneys after 24 hours (Fig. 2C and D, Table 1) and 48 hours (Table 1) of reperfusion. Bromocriptine did not produce any detectable histologic

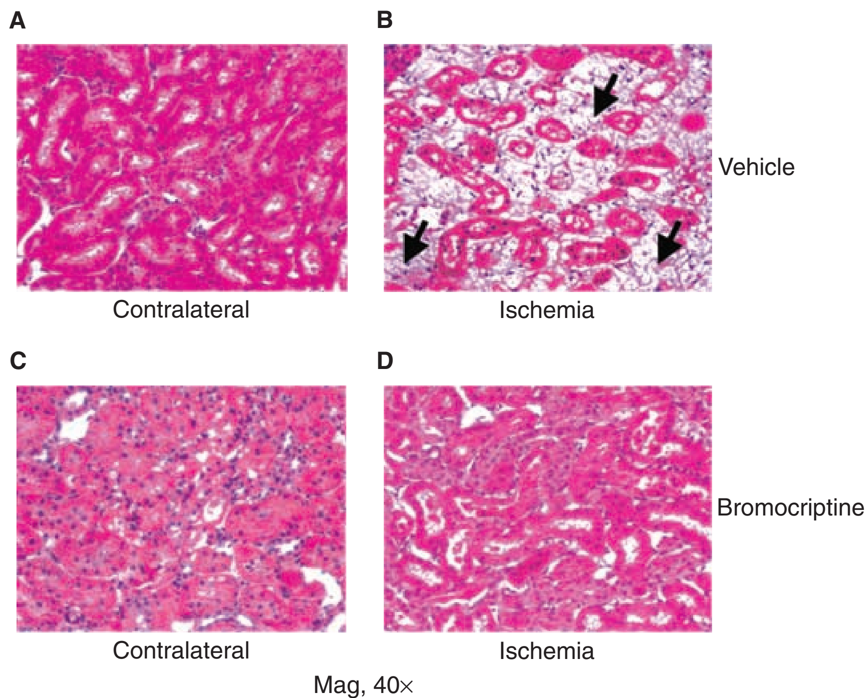
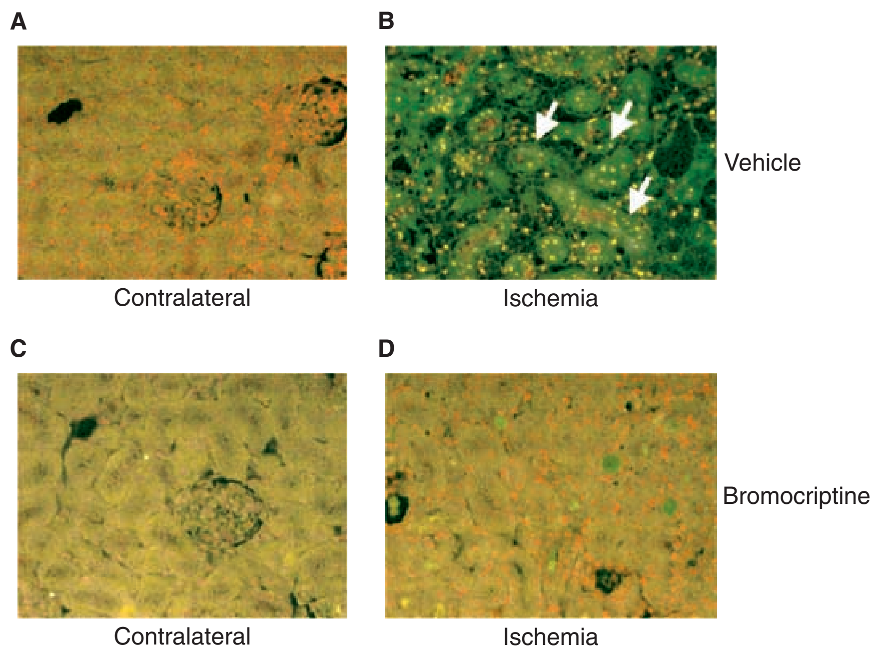


Fig. 2. Histology in cortical region of kidney slices of unilateral I/R rat model. Representative light micrographs (magnification, 40×) of hematoxylin and eosin stained sections showing morphologic features of cortical region 24 hours post I/R injury. In vehicle-treated rats, the ischemic cortical slices (*B*) showed extensive necrosis (indicated by arrows) compared to the contralateral kidney (*A*). The histomorphology of bromocriptine-treated ischemic kidneys (*D*) was normal and indistinguishable from contralateral control kidneys (*C*). Similar results were obtained from 5 animals.



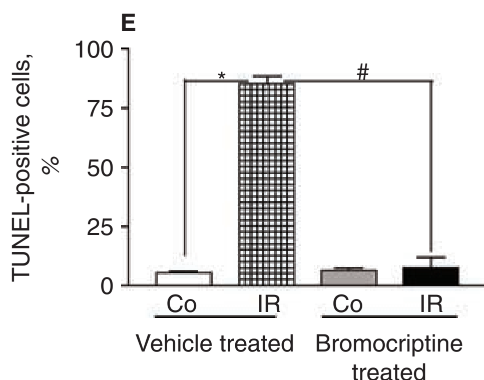


Fig. 3. Apoptosis in cortical region of kidney slices of unilateral ischemic/reperfusion (I/R) injury rat model. Apoptosis was determined in 24 hours post-I/R cortical kidney slices. Compared to the contralateral cortical slices (*A*), in vehicle-treated rats, the ischemic cortical slices (*B*) showed extensive TUNEL-positive staining. (*C*) The contralateral cortical slices of bromocriptine-treated rats are represented. In bromocriptine-treated rats low TUNEL-positive staining was detected in the ischemic cortical slices (*D*). (*E*) Data represented as % TUNEL-positive cells ($N = 3$). ∗ and # represent statistically significant difference between groups compared ($P < 0.05$) [one-way analysis of variance (ANOVA); post hoc, Tukey's multiple comparision test].

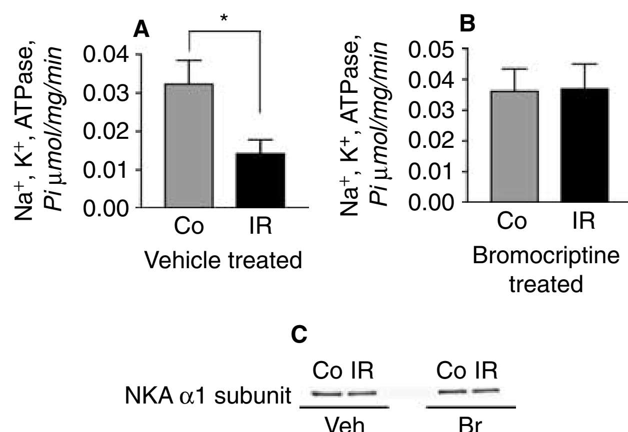


Fig. 4. Detection of NKA activity and α1 subunits in renal cortical homogenates of unilateral ischemic/reperfusion (I/R) rat model. NKA activity and α1 subunit levels were determined in cortical homogenates obtained from the vehicle-treated and bromocriptine-treated rats 24 hours post-I/R. (*A*) In vehicle-treated rats, the NKA activity in ischemic cortical homogenate (IR) was significantly lower than in the contralateral cortical homogenate (Co). (*B*) In bromocriptine-treated rats, NKA activity was similar between contralateral and ischemic cortical homogenates. Data are represented as inorganic phosphate (Pi) released in µmol/mg/min. *Represents significant difference between Co and IR in vehicle-treated rats ($P < 0.05$) (unpaired Student *t* test). (*C*) Representative Western blot of NKA α1 subunit expression in cortical homogenates of vehicle-treated and bromocriptine-treated rats 24 hours post I/R. Similar results were obtained in $N = 4$ animals.

abnormalities in the contralateral kidney. This result indicates that bromocriptine prevented cortical necrosis in renal I/R injury after 24 and 48 hours post-reperfusion.

Apoptosis in the cortical slices of vehicle- and bromocriptine-treated rats

Apoptosis was detected by TUNEL assay performed in the cortical slices from the contralateral and ischemic kidneys. Extensive TUNEL-positive staining was observed in the outer cortex of ischemic compared to contralateral kidney of vehicle-treated rats (Fig. 3A and B). In contrast, low level of TUNEL-positive staining was detected in contralateral and ischemic kidneys of bromocriptine-treated rats (Fig. 3C and D). The percent of cells that were TUNEL-positive in each group is represented in Figure 3E. This result indicates that bromocriptine prevented cortical apoptosis in renal I/R injury.

NKA activity in the cortical homogenates of vehicle- and bromocriptine-treated rats

NKA activity was measured as described in the cortical homogenates of the kidney. It was found that the NKA activity in the cortical homogenate from the ischemic kidney was decreased as compared to the contralateral kidney in vehicle-treated rats (Fig. 4A). NKA activity in the bromocriptine-treated rats was same in both the contralateral and the ischemic kidney. Moreover, this activity was similar to that of the contralateral kidney of vehicle-treated rats (Fig. 4B). This result indicates that bromocriptine prevents the loss of NKA activity in the cortex of the ischemic kidney.

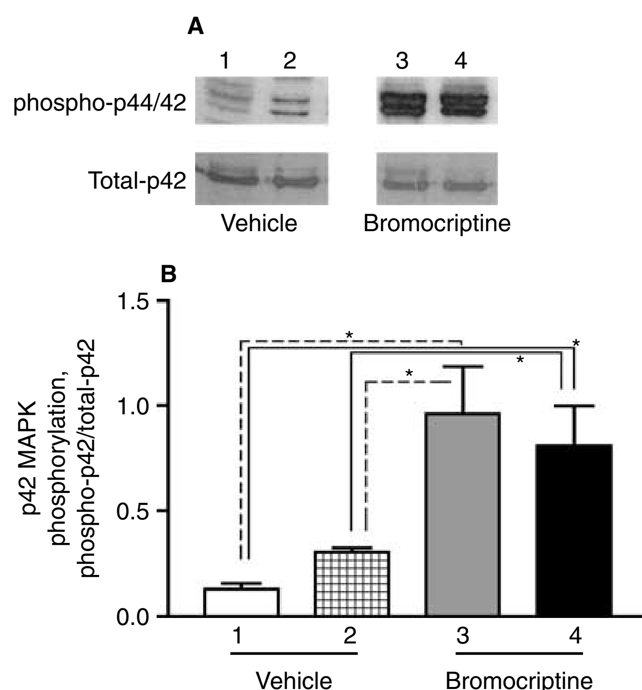


Fig. 5. Determination of p44/42 mitogen-activated protein kinases (MAPKs) phosphorylation in renal cortical homogenates of unilateral ischemic/reperfusion (I/R) rat model. (*A*) In vehicle-treated rats, low phosphorylation was detected in contralateral (1) and ischemic (2) cortical homogenate. On the other hand, bromocriptine-treated rats showed extensive phosphorylation in contralateral (3) and ischemic (4) cortical homogenate (upper panel). The total-p42 MAPKs was similar in all the groups compared (1, 2, 3, and 4) (lower panel). The above are representative blots. Similar results were obtained from $N = 4$ sets of animals. (*B*) Data represented as average (mean ± SEM) ratio of phospho-p42 to total p42 MAPKs from $N = 4$ animals. ($P < 0.05$) [one-way analysis of variance (ANOVA); post hoc, Newman-Keuls multiple comparison test).

To test the possibility that change in the NKA activity is due to modified protein levels, we measured the expression of catalytic NKA α1 subunit in the above homogenates. We did not detect any difference in the expression of NKA α1 subunits in the contralateral or ischemic cortical homogenates from vehicle and bromocriptine-treated animals (Fig 4C). Therefore, the changes in NKA activity are independent of the level of NKA α1 subunits in the cortex.

The p44/42 MAPKs activity in the vehicle- and bromocriptine-treated rats

The p44/42 MAPKs activity was measured as the function of p44/42 MAPKs phosphorylation in the cortical homogenates of the kidney. In the vehicle-treated rats, there was low detectable phosphorylation of p44/42 MAPKs in both the control and ischemic cortices (Fig. 5A, upper panel). The total p42 MAPKs in all the homogenates

was similar (Fig. 5A, lower panel). In bromocriptine-treated rats the phosphorylation of p44/42 MAPKs was similar in both the contralateral and ischemic kidney (Fig. 5A, upper panel). Moreover, the phosphorylation of p44/42 MAPKs in the bromocriptine-treated rats was significantly higher than the phosphorylation in the vehicle-treated rats. This was not simply due to more p44/42 MAPKs protein, as the total protein loaded was same in vehicle-treated and bromocriptine-treated rats (Fig. 5A, lower panel). Thus, bromocriptine increases phospho-p44/42 MAPKs in both contralateral and ischemic cortices of bromocriptine-treated rats as compared to vehicle-treated rats. The above data are represented as densitometric ratio of phospho-p42 to total-p42 MAPKs (Fig. 5B).

DISCUSSION

This study was designed to explore the potential of synthetic dopamine D_2-like receptor agonist, bromocriptine, to protect against experimental I/R injury to the renal proximal tubules. We found that I/R injury caused necrosis, apoptosis, and loss of NKA activity in the outer cortex of the kidney, which is rich in proximal tubules. Interestingly, pretreatment of the animals with bromocriptine prevented necrosis, apoptosis, and loss of NKA activity associated with I/R injury in proximal tubular regions of the kidney. This protective effect may be due to the activation of p44/42 MAPKs in the bromocriptine-treated kidney.

I/R injury is known to induce differential activation of various MAPKs in different segments of the kidney nephron [10]. Thus, selective activation of JNK leads to apoptosis of tubular cells, whereas activation of p44/42 MAPKs renders the cells viable [10]. It was suggested that I/R injury leads to activation of JNK and not p44/42 MAPKs in proximal tubules, making them more susceptible to apoptosis [10, 17]. In another study, a brief ischemic preconditioning [4] to kidney or urethral obstruction [5] prior to a second ischemia protected the kidney morphologically, as well as functionally against I/R injury. In these studies it was found that either pretreatment primes the kidney such that, during the second I/R injury there is decreased p38/JNK activation and/or increased p44/42 MAPKs stimulation. Alternatively, pharmacologic interventions which protect against renal I/R injury also increase p44/42 MAPKs in the kidney [10, 17]. Since bromocriptine activates p44/42 MAPKs in renal proximal tubular cells [21, 22], which are known to be maximally susceptible to I/R injury, it was logical to study the effects of bromocriptine in this pathophysiologic situation.

We measured the effect of bromocriptine pretreatment on I/R injury–induced necrosis, apoptosis, and downregulation of NKA activity in the proximal tubules of the kidney. We found that unilateral I/R injury to kidney caused extensive necrosis by 24 hours in the cortex (rich in proximal tubules) of ischemic kidney as compared to the control kidney. This observation was in concert with previous reports [8, 17, 23]. When the animals were pretreated with bromocriptine, the kidney did not undergo necrosis, indicating the protective effect of bromocriptine.

In addition to necrosis, unilateral I/R injury caused apoptosis in the cortex of the ischemic kidney as compared to the control kidney in the vehicle-treated rats. Pretreatment of the rats with bromocriptine prevented I/R injury–induced apoptosis in the kidney. This protective effect of bromocriptine can be attributed to activation of p44/42 MAPKs. As mentioned above, JNK is predominantly activated in the cortex during I/R injury, which predisposes the proximal tubules to apoptotic death [10]. By activating p44/42 MAPKs, which are anti-apoptotic, bromocriptine might cancel out or balance the effect of JNK.

In concert with some previous reports [24, 25], we found that the NKA activity in the ischemic cortex was decreased as compared to the contralateral nonischemic cortex. However, our results contradict alternative studies which report no change or actual increase in the NKA activity in the kidney [26, 27]. This discrepancy may be associated with the differences in the models of I/R injury used or due to the use of whole kidney lysate versus cortical lysates for measuring NKA activity. Nevertheless, pretreatment of the animals with bromocriptine in our experiments prevented the loss of NKA activity in I/R injury. Unlike the NKA activity, the expression of catalytic NKA $\alpha1$ subunit remained constant in all the treatment groups. The consistent expression of NKA $\alpha1$ subunit suggests that bromocriptine restores NKA activity without changing the protein expression. Interestingly, earlier studies done in our laboratory show that bromocriptine stimulates NKA activity via p44/42 MAPKs-dependent recruitment of NKA $\alpha1$ subunits to the proximal tubular membrane in the kidney [21, 22, 28, 29]. It is possible that bromocriptine may prevent the loss of NKA activity in renal I/R injury by similar mechanism. Moreover, maintenance of NKA activity in the proximal tubular membrane may partially prevent the increase in fractional excretion of sodium observed during I/R injury [2].

As previously reported, we found that there was lower phospho (active)-p44/42 MAPKs in ischemic cortex of vehicle-treated animals [10]. On the other hand, robust increase in phospho-p44/42 MAPKs was observed in both the ischemic and the control non-ischemic kidneys of bromocriptine-treated animals. Further, activation of p44/42 MAPKs in the bromocriptine-treated animals were concomitant with the protection against I/R injury (at the level of necrosis, apoptosis, and NKA activity) in the same animals. In terms of proapoptotic

MAPKs, several drugs protect against I/R injury by decreasing JNK activity in addition to increasing that of p44/42 MAPK [10, 17, 30–33]. However, in experiments done in our lab, bromocriptine did not decrease the elevated JNK activity in the ischemic cortex (data not shown). Therefore, bromocriptine may protect against renal I/R injury by increasing antiapoptotic p44/42 MAPKs activity, without additionally modifying proapoptotic JNK activity.

It is noteworthy that certain studies suggest the increased renal phospho-p44/42 MAPKs to be actually responsible for I/R injury [31, 34]. Moreover, in the same studies reno-protective agents such as nitric oxide donors protect against renal I/R injury and simultaneously decrease phospho-p44/42 MAPKs in the ischemic kidneys [31, 34]. At this point it is hard to explain the discrepancy between these reports and our observations. However, there are several differences between our experiments and ones performed in these studies at levels such as the model of I/R injury and time of phospho-p44/42 MAPK measurement, which can account for different observations. Moreover, as mentioned above, we detected increased phospho-p44/42 MAPKs in the control kidney of bromocriptine-treated animals, which showed no signs of necrosis or apoptosis. Therefore, it is more likely that p44/42 MAPKs are protective rather than causative in renal I/R injury.

The activation of p44/42 MAPKs may not be the sole mechanism for the protective effects of bromocriptine. It is interesting to note that the protective effects of bromocriptine and other dopamine D_2-like receptor agonist against cerebral I/R injury have been previously reported [35, 36, 37]. Multiple mechanisms such as (*1*) inhibition of dopamine release [38], (*2*) activation of superoxide dismutase/free radical scavenging [39, 40, 41, 42, 43], and (*3*) activation of antiapoptotic factors [35] are responsible for neuroprotective effects of dopamine D_2-like receptor agonists. It is likely that similar mechanisms, in addition to the one we have described, may also play a role in the protection of proximal tubules during I/R injury.

CONCLUSION

Our results show that bromocriptine, a dopamine D_2-like receptor agonist, protects against renal I/R injury. Further, this protective effect may primarily involve activation of p44/42 MAPK. Additional studies are necessary to explore the potential of bromocriptine in treating post-I/R acute renal failure.

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