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Activation of Dopamine D_4 Receptors Is Protective Against Hypoxia/Reoxygenation-Induced Cell Death in HT22 Cells

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Abstract. Several reports have shown that some dopamine receptor ligands modulate the ischemia–reperfusion injury in animal models; however, its underling mechanisms are still unclear. In this study, we sought to establish an in vitro experimental model of hypoxia/reoxygenation (H/R) using HT22 cells that originated from mouse hippocampal neurons and to examine protective the effect of dopamine-receptor ligands against H/R-induced cell injury. The treatment with hypoxia for 18 h followed by reoxygenation for 6 h induced the elevation of intracellular reactive oxygen species (ROS) and reduction of mitochondrial membrane potential; however, lactate dehydrogenase (LDH) release was not changed at this time point. LDH release was increased after reoxygenation for 18 h and longer, and this increase in LDH release was suppressed by dopamine receptor agonists such as apomorphine and apocodeine. The suppressive effects of these agonists were reversibly inhibited by L750667, a D_4-receptor antagonist but not by D_2- or D_3-receptor antagonists. In addition, PD168077, a selective dopamine D_4–receptor agonist, also protected against H/R-induced cell death. These results suggest that H/R causes oxidative stress–induced cell death and that the activation of dopamine D_4 receptors protects against H/R-induced cell death in HT22 cells.

Keywords: dopamine D_4 receptor, hypoxia/reoxygenation, apomorphine, oxidative stress, cell death

Introduction

Brain ischemia/reperfusion (I/R) injury is the leading cause of neuronal cell death. The neuronal damage induced by brain ischemia results in functional impairment and death. Accumulated data have led to a better understanding of the pathophysiology of brain I/R injury (1 – 6), and it has become clearer that I/R injury is, in part, related to an increase in the generation of reactive oxygen species (ROS) after reperfusion (6 – 8). It has also been demonstrated that markers of oxidative stress, such as superoxide dismutase and 4-hydroxynonenal, are increased after reperfusion (1 – 3). Edaravone, a radical scavenger, has been approved by Japanese health authorities as a neuroprotective agent for the treatment of acute cerebral infarction since 2001 (9). Edaravone suppresses cortical edema, reduces the infarct volume, and improves neurological deficits in rats with cerebral ischemia (10, 11). In addition to edaravone, several drugs, such as tissue plasminogen activator and aspirin (12) have been used for the treatment of brain I/R injury. However, currently available therapeutic drugs for brain ischemia are limited, and development of additional agents is needed.

Several reports have indicated that some dopamine-receptor agonists modulate I/R injury in animal models (13, 14). SCH23390, a dopamine D_1–like receptor agonist, attenuates ischemia-induced cell death in the striatum of rats (14). Pergolide, a dopamine D_2–receptor agonist, has been shown to protect hippocampal CA1 neurons against injury after reperfusion in a global cerebral ischemia model in the gerbil (13). Bromocriptine, a D_2-like receptor agonist, protects kidney cells against I/R-induced injury in rats (15). It has also been demonstrated that

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apomorphine, a non-selective dopamine-receptor agonist (16), prevents myocardial I/R–induced oxidative stress in rat heart and that this protective effect is due to an anti-oxidation mechanism (17, 18). In addition, our previous study demonstrated that apomorphine protected HT22 cells, originally derived from mouse hippocampal neurons, against oxidative stress–induced cell death through the activation of dopamine D_4 receptors (19). In contrast, it has been suggested that L745870, a dopamine D_4–receptor antagonist, attenuates ischemia-induced neuronal cell damage in the hippocampal CA1 area in gerbils (20). These data suggest that certain dopamine-receptor ligands exert protective effects against neuronal injury; however, the underling mechanisms are still unclear. Recently, in order to investigate the mechanisms involved in I/R-induced cell death and novel compounds that might be effective for treatment of I/R injury, cultured cells exposed to hypoxia–reoxygenation (H/R) have been used as an in vitro model of I/R injury (21, 22). In the present study, we sought to establish an in vitro experimental condition of H/R injury in HT22 cells and used it to examine the effects of dopamine receptor ligands on H/R-induced cell death.

Materials and Methods

Materials

The chemicals used were Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), trypsin 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA), and propidium iodide (PI) (Invitrogen, Carlsbad, CA, USA); apomorphine, apocodeine, PD168077, L741626, L750667, and nafadotride (Sigma-Aldrich, Inc., St. Louis, MO, USA); lactate dehydrogenase (LDH) cytotoxic test and trypan blue (Wako Pure Chemical Industries, Osaka); MitoRed (Dojindo Laboratories, Kumamoto); and AnaeroPack for cell^{TM} (Mitsubishi Gas Chemical Co., Tokyo).

Cell culture and H/R

HT22 cells were maintained at 37°C in a humidified atmosphere containing 10% CO_2 in DMEM supplemented with 10% FBS. Cells were exposed to hypoxia using AnaeroPack for cell^{TM}. After exposure to hypoxia for the indicated times, the cells were returned to normal atmosphere (reoxygenation).

LDH release assay

HT22 cells were seeded onto 96-well microtiter plates at a density of 1×10^2 cells/well in 100 μL of DMEM supplemented with 10% FBS. The next day, cells were treated with H/R. At the end of the reoxygenation period, the amount of LDH released by cells was determined by a LDH cytotoxic test kit according to manufacturer's protocol. The absorbance of samples was measured at 570 nm. The results were shown as the percentage against the control value specified in each experiment. In all cases, cell death was confirmed by microscopy.

Trypan blue exclusion test

HT22 cells were seeded onto 6-well plates at a density of 1×10^4 cells/well, similar to the LDH release assay. After the indicated treatments, cells were harvested using a cell scraper and collected by centrifugation. The cells were resuspended in 100 μL DMEM without phenol red and 10 μL trypan blue solution (0.4%) was added. The cells that did not exclude the trypan blue dye were considered dead. The percentage of dead cells (trypan blue–positive cells) against the total number of cells was calculated.

Assessment of cell viability (Hoechst 33258 / PI staining)

Cell viability was determined by a double-staining procedure using Hoechst 33258 / PI as reported previously (23). The stained cells were examined immediately with a standard epi-illumination fluorescence microscope. Cells that were positively stained with PI were considered to be dead.

Measurement of intracellular ROS

ROS levels were determined by a staining procedure using H_2DCFDA. HT22 cells were seeded onto 6-well plates similar to the trypan blue exclusion test. After the indicated treatments, cultured medium were removed and incubated for 15 min with H_2DCFDA (5 μM) in DMEM without phenol red. The cells were then washed and ROS was visualized by fluorescence microscopy (480 nm/526 nm). Fluorescence of randomly selected cells was measured with Scion Image Software (Scion Co., Frederick, MD, USA).

Measurement of mitochondrial membrane potential

Mitochondrial membrane potential was determined by a staining procedure using MitoRed. HT22 cells were seeded onto 6-well plates similar to trypan blue exclusion test. After the indicated treatments, cultured medium were removed, and incubated for 30 min with MitoRed (1 μM) in DMEM without phenol red. The cells were washed, fixed, and then visualized by fluorescence microscopy (560 nm/580 nm). Fluorescence of randomly selected cells was measured with Scion Image Software.

Statistical analysis

Values are expressed as the mean ± S.E.M. The sig-

nificance of differences between two groups was assessed by Student's *t*-test. Statistical significance among more than three groups was assessed with one-way analysis of variance followed by Tukey's multiple range test. $P < 0.05$ was considered significant.

Results

H/R-induced injury in HT22 cells

In order to establish an in vitro experimental model of H/R-induced cell death in HT22 cells, cell death in hypoxic cells was compared with that in normoxic cells. Treatment with hypoxia for 18 h followed by reoxygenation for 24 h (H18/R24) decreased the total number of cells and increased the proportion of PI-positive cells (Fig. 1A). Hypoxic stimulation without reoxygenation did not affect LDH release in these cells for up to 24 h (Fig. 1B). In contrast, LDH release was found to be dependent on the duration of hypoxia (6 – 24 h) when examined 24 h after reoxygenation (Fig. 1C). In addition, LDH release and the number of trypan blue–positive cells were dependent on the duration of reoxygenation (6 – 24 h) when the cells were subjected to a fixed period of hypoxia (18 h) beforehand (Fig. 1D, E).

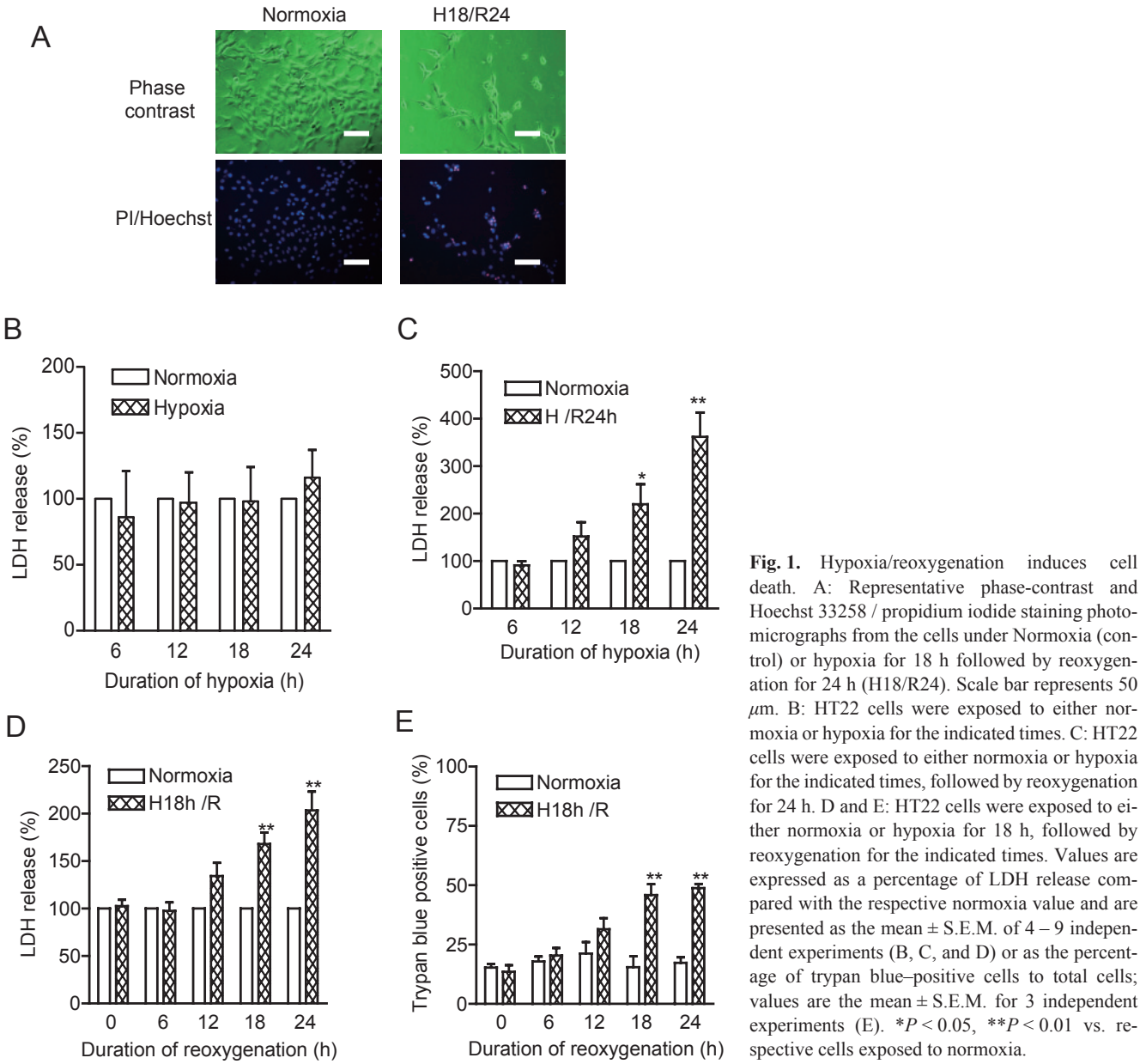

Fig. 1. Hypoxia/reoxygenation induces cell death. A: Representative phase-contrast and Hoechst 33258 / propidium iodide staining photomicrographs from the cells under Normoxia (control) or hypoxia for 18 h followed by reoxygenation for 24 h (H18/R24). Scale bar represents 50 μm. B: HT22 cells were exposed to either normoxia or hypoxia for the indicated times. C: HT22 cells were exposed to either normoxia or hypoxia for the indicated times, followed by reoxygenation for 24 h. D and E: HT22 cells were exposed to either normoxia or hypoxia for 18 h, followed by reoxygenation for the indicated times. Values are expressed as a percentage of LDH release compared with the respective normoxia value and are presented as the mean ± S.E.M. of 4 – 9 independent experiments (B, C, and D) or as the percentage of trypan blue–positive cells to total cells; values are the mean ± S.E.M. for 3 independent experiments (E). $^*P < 0.05$, $^{**}P < 0.01$ vs. respective cells exposed to normoxia.

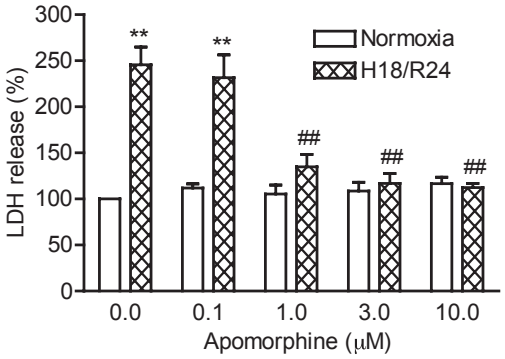

Fig. 2. Protective effect of apomorphine against H/R-induced cell death. HT22 cells were incubated with apomorphine 12 h before exposure to hypoxia for 18 h, followed by reoxygenation for 24 h (H18/R24). Cell death was measured by LDH release assay. Values are expressed as a percentage of LDH release compared with that in the control (normoxia without apomorphine) cells, and are presented as the mean ± S.E.M. for 7 – 10 independent experiments. $^{**}P < 0.01$ vs. respective values for normoxia, $^{\#\#}P < 0.01$ vs. H18/R24 without apomorphine.

Protective effect of apomorphine against H/R-induced cell death

Treatment of the cells with apomorphine (0.1 – 10 μM), a dopamine-receptor agonist, suppressed the H18/R24-induced increase of LDH release in a concentration-dependent manner (Fig. 2), and treatment with 3 or 10 μM apomorphine attenuated the H18/R24-induced increase of LDH to the control level. The protective effect of apomorphine (3 μM) was completely inhibited by L750667 (3 μM), a dopamine D_4–receptor antagonist (Fig. 3A). Another dopamine-receptor agonist, apocodeine (3 μM), also suppressed the H/R-induced increase in LDH release, and L750667 (3 μM) inhibited this protective effect (Fig. 3B). In contrast, L741626 (3 μM), a dopamine D_2–receptor antagonist, and nafadotride (3 μM), a dopamine D_3–receptor antagonist, did not alter the protective effect of apomorphine at the same concentration as L750667 (Fig. 3C, D). In addition, PD168077 (5 – 100 μM), a selective dopamine D_4 agonist, suppressed the H/R-induced increase of LDH release in a concentration-dependent manner (Fig. 4).

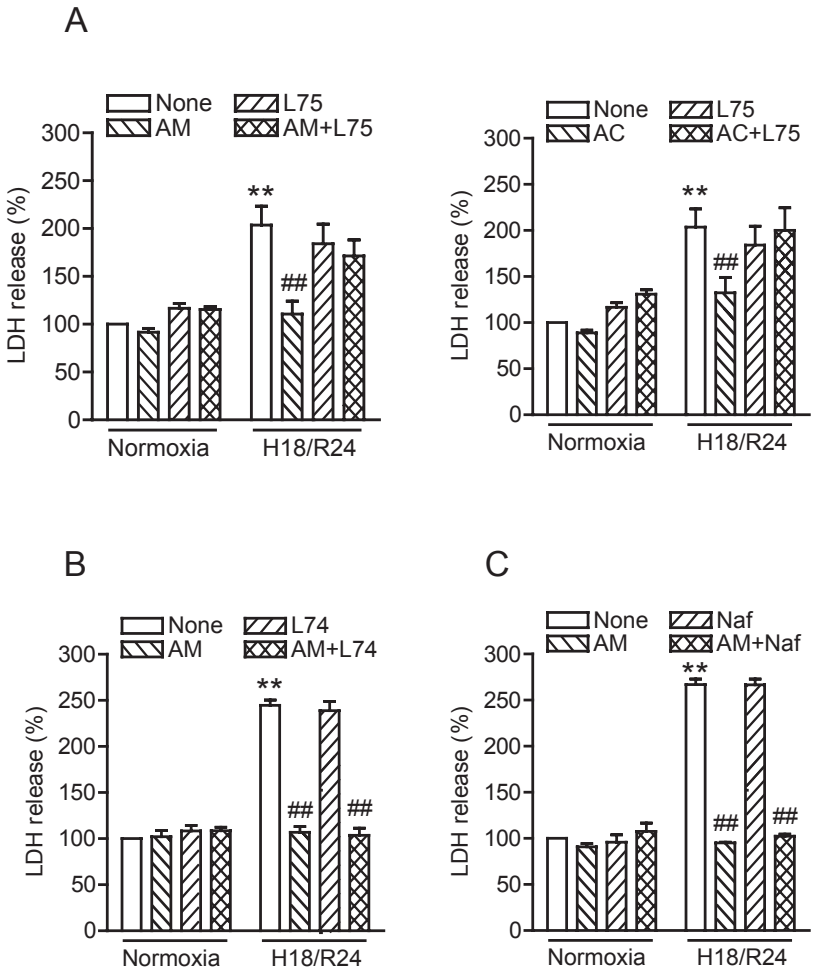

Fig. 3. Role of dopamine D_4 receptors in the protective effects of apomorphine and apocodeine against H/R-induced cell death. HT22 cells were incubated with apomorphine (AM, 3 μM) or apocodeine (AC, 3 μM) with or without dopamine receptor antagonists (3 μM) 12 h before exposure to hypoxia for 18 h, followed by reoxygenation for 24 h (H18/R24). Cell death was measured using the LDH release assay. Values are expressed as the percentage of LDH release compared with that in cells exposed to normoxia, and are presented as the mean ± S.E.M. for 3 – 7 independent experiments. $^{**}P < 0.01$ vs. control cells (normoxia, None), $^{\#\#}P < 0.01$ vs. H18/R24 without dopamine receptor ligands. The following dopamine antagonists were used: A: L750667 (L75, 3 μM), B: L741626 (L74, 3 μM), C: nafadotride (Naf, 3 μM).

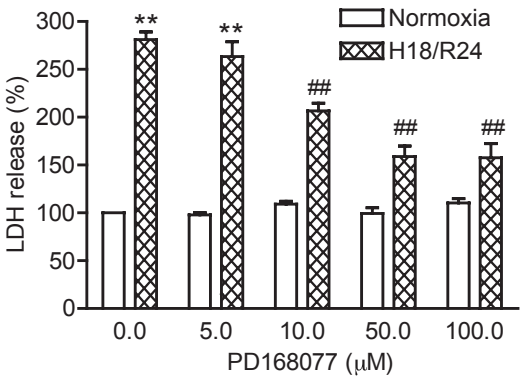

Fig. 4. Protective effect of PD168077, a dopamine D_4 agonist, against H/R-induced cell death. HT22 cells were incubated with PD168077 12 h before exposure to hypoxia for 18 h, followed by reoxygenation for 24 h (H18/R24). Cell death was measured by LDH release assay. Values are expressed as the percentage of LDH release compared with that in cells exposed to normoxia and are presented as the mean ± S.E.M. for 7 – 10 independent experiments. $^{**}P < 0.01$ vs. respective values for normoxia, $^{\#\#}P < 0.01$ vs. H18/R24 without PD168077.

Intracellular ROS levels and mitochondrial membrane potential

It has been suggested that the intracellular ROS level is increased by reoxygenation and that oxidative stress induces a loss of mitochondrial membrane potential. As shown in Fig. 5, intracellular ROS levels were increased transiently, with a peak level at 6 h of reoxygenation. Treatment with apomorphine (3 μM) completely suppressed the increase in intracellular ROS levels induced by reoxygenation (Fig. 5). In addition, mitochondrial membrane potentials, measured by MitoRed, were lowered by reoxygenation in a time-dependent manner (Fig. 6). Apomorphine (3 μM) also suppressed the H/R-induced reduction of the mitochondrial membrane potential (Fig. 6).

Discussion

It has been suggested that ROS are generated during I/R and H/R and that I/R- and H/R-induced injuries are, in part, related to the burst of ROS generation after reperfusion (6 – 8, 24).

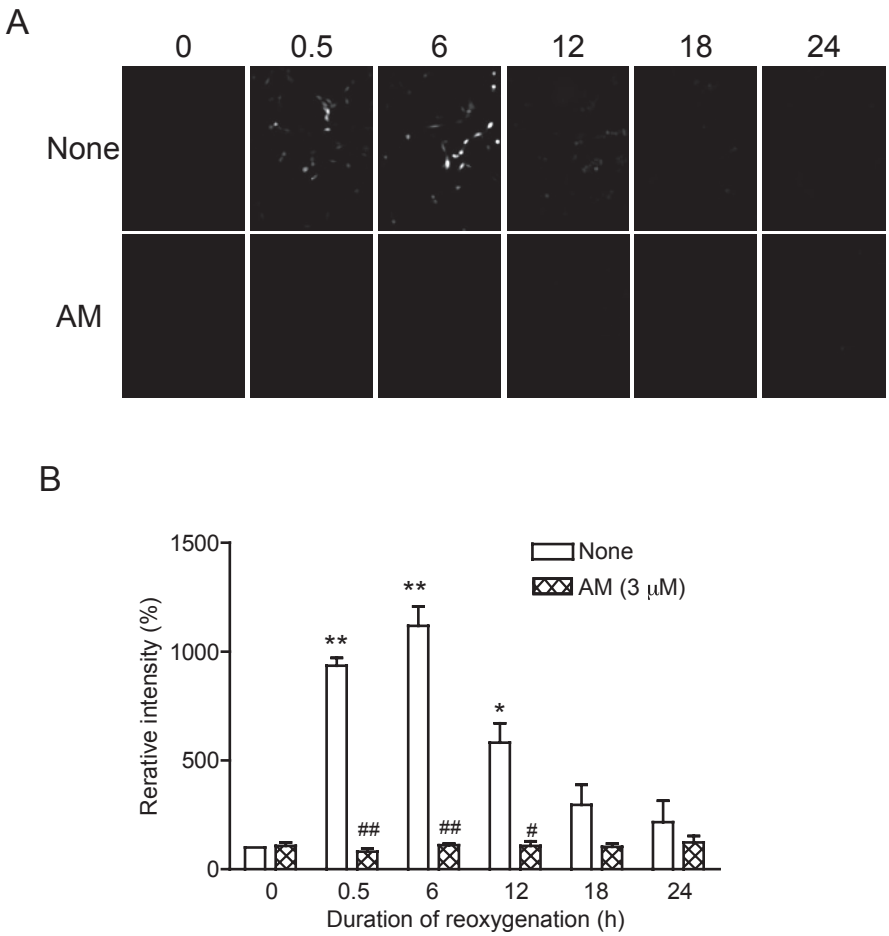

Fig. 5. Effect of apomorphine on the H/R-induced increase in intracellular reactive oxygen species (ROS). HT22 cells were incubated with apomorphine (AM, 3 μM) 12 h before exposure to hypoxia. After exposure to hypoxia for 18 h followed by reoxygenation for the indicated times, ROS levels were measured as described in Materials and Methods. A: Representative photographs of time course change in fluorescence. B: Fluorescence in each experiment was calculated from 15 randomly selected cells. Values are presented as the mean ± S.E.M. for 3 independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control cells (none, 0); $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. respective none.

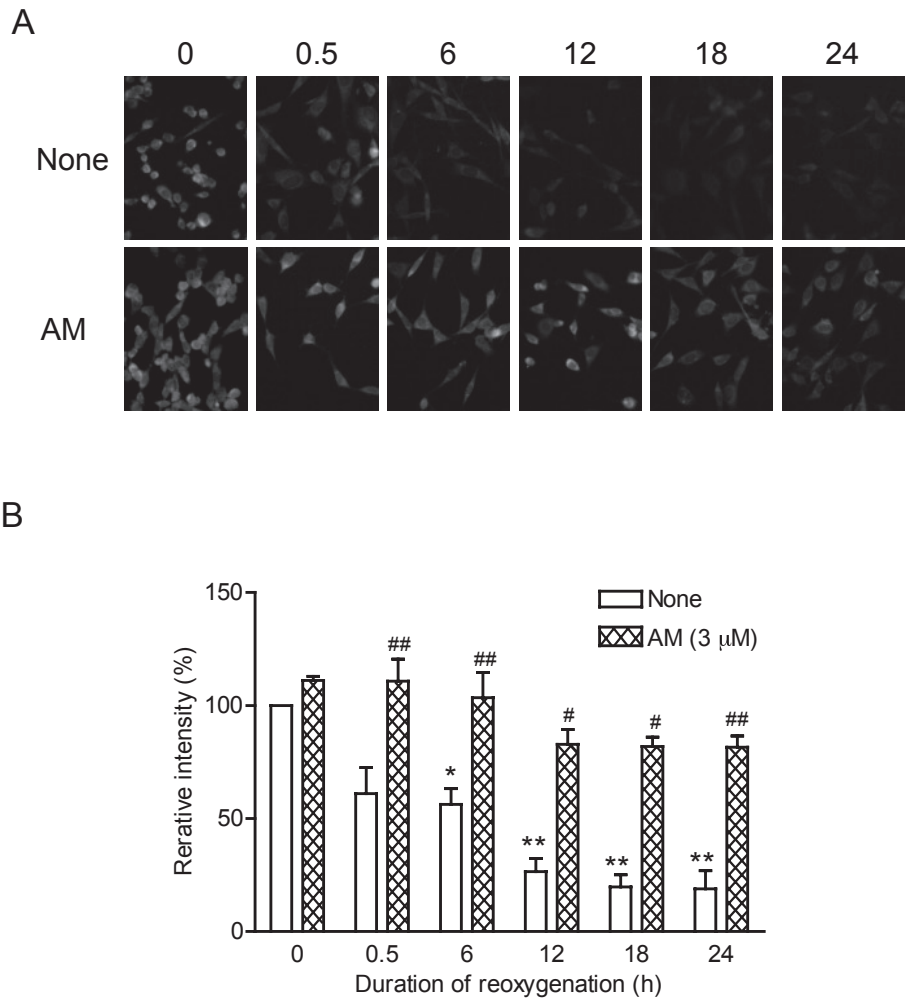

Fig. 6. Effect of apomorphine on mitochondrial membrane potential. HT22 cells were incubated with apomorphine (AM, 3 μM) 12 h before exposure to hypoxia. After exposure to hypoxia for 18 h followed by reoxygenation for the indicated times, mitochondrial membrane potential was measured as described in Materials and Methods. A: Representative photographs of time course change in fluorescence. B: Fluorescence in each experiment was calculated from 15 randomly selected cells. Values are presented as the mean ± S.E.M. for 3 independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control cells (none, 0), $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. respective none.

The data presented here demonstrated that hypoxic stimulation alone for 6 – 24 h did not affect the survival of HT22 cells; however, reoxygenation followed by hypoxia induced cell death in a time-dependent manner (Fig. 1). In addition, a transient increase in ROS and a reduction of mitochondrial membrane potential were also observed after exposure to hypoxia followed by reoxygenation (Figs. 5 and 6). Taken together with previous evidence (6 – 8, 24), these results suggest that ROS play a pivotal role in H/R-induced death in HT22 cells. Thus, induction of H/R-induced death in HT22 cells was at least partly due to activation of the oxidative stress pathway.

Our previous study demonstrated that activation of dopamine D_4 receptors protected HT22 cells against death induced by glutamate-induced oxidative stress (19). It has been demonstrated that dopamine D_4 receptors are expressed in HT22 cells and that agonists such as apomorphine and apocodeine bind to the D_4 receptors with high affinity. The present study demonstrated clearly that treatment of HT22 cells with apomorphine and apocodeine 12 h before exposure to hypoxia suppressed cell death as measured by LDH release, the transient increase in ROS, and the decrease in mitochondrial membrane potential induced by H/R and that L750667, a dopamine D_4 antagonist (25), reversibly antagonized the protective effects of these agonists. The administration of the D_4 agonists just before reoxygenation also decreased ROS induction and protected against cell death (data not shown). In contrast, neither L741626, a D_2 antagonist (26), nor nafadotride, a D_3 antagonist (27), altered the protective effect of apomorphine against H/R-induced cell death. In addition, PD168077, a selective dopamine D_4–receptor agonist (28), also suppressed H/R-induced cell death; however, the protective effect of this compound was weaker than that of apomorphine, suggesting that the former has lower affinity for D_4 receptors than the latter (19). It has also been shown that the concentration of apomorphine required for suppression of cell death is also lower than that of PD168044 in glutamate-induced cell death (19).

Suppression of the H/R-induced increase in ROS and

loss of mitochondrial membrane potential by apomorphine suggested that this compound is an effective radical scavenger. It has also been shown that apomorphine-induced myocardial protection is attributable to its antioxidant effect and not via dopamine receptors (17, 18). Therefore, it seems that the antioxidant properties of apomorphine might be responsible for its protective effect against ROS-induced injury. However, our previous study demonstrated that D_4 receptor–mediated mechanisms are more predominant than the antioxidant activity of apomorphine in protecting against glutamate-induced oxidative cell death in HT22 cells (19). In HT22 cells, an increase of ROS induces Ca^{2+} influx through cGMP-gated Ca^{2+} channels (19, 29 – 32), and this elevation of the intracellular Ca^{2+} level is critical for oxidative stress–induced cell death. Indeed, apomorphine completely inhibited the glutamate-induced increase of intracellular Ca^{2+} and cell death; however, the increase in ROS was only partially inhibited (19). In addition, the antioxidant activity of apomorphine estimated in terms of trolox equivalent antioxidant capacity (TEAC) value is higher than that of apocodeine, whereas there is no difference in the protective effect against glutamate-induced cell death between the two. Although PD168077 had no antioxidant activity, glutamate-induced cell death was prevented by PD168077 (19). In the present study, PD168077 also protected cells against H/R-induced toxicity. Therefore, it is very likely that apomorphine and other D_4 agonists inhibit ROS elevation downstream in the H/R-induced cell death pathway. Details of this pathway, including the intracellular Ca^{2+} level, remain to be clarified further.

In conclusion, the present data obtained using HT22 cells have demonstrated that H/R causes oxidative stress–induced cell death and that activation of dopamine D_4 receptors has a protective effect against H/R-induced cell death.

References

1 Saeed SA, Shad KF, Saleem T, Javed F, Khan MU. Some new prospects in the understanding of the molecular basis of the pathogenesis of stroke. Exp Brain Res. 2007;182:1–10.

2 Matsuda S, Umeda M, Uchida H, Kato H, Araki T. Alterations of oxidative stress markers and apoptosis markers in the striatum after transient focal cerebral ischemia in rats. J Neural Transm. 2009;116:395–404.

3 Kishi T, Hirooka Y, Kimura Y, Ito K, Shimokawa H, Takeshita A. Increased reactive oxygen species in rostral ventrolateral medulla contribute to neural mechanisms of hypertension in stroke-prone spontaneously hypertensive rats. Circulation. 2004;109:2357–2362.

4 Meloni BP, Campbell K, Zhu H, Knuckey NW. In search of clinical neuroprotection after brain ischemia: the case for mild hypothermia (35 degrees C) and magnesium. Stroke. 2009;40:2236–2240.

5 Osada N, Kosuge Y, Kihara T, Ishige K, Ito Y. Apolipoprotein E-deficient mice are more vulnerable to ER stress after transient forebrain ischemia. Neurochem Int. 2009;54:403–409.

6 Medling BD, Bueno R, Chambers C, Neumeister MW. The effect of vitamin E succinate on ischemia reperfusion injury. Hand. 2009;5:60–64.

7 Hashimoto T, Yonetani M, Nakamura H. Selective brain hypothermia protects against hypoxic-ischemic injury in newborn rats by reducing hydroxyl radical production. Kobe J Med Sci. 2003;49:83–91.

8 Dhar-Mascareno M, Carcamo JM, Golde DW. Hypoxia-reoxygenation-induced mitochondrial damage and apoptosis in human endothelial cells are inhibited by vitamin C. Free Radic Biol Med. 2005;38:1311–1322.

9 Zhang N, Komine-Kobayashi M, Tanaka R, Liu M, Mizuno Y, Urabe T. Edaravone reduces early accumulation of oxidative products and sequential inflammatory responses after transient focal ischemia in mice brain. Stroke. 2005;36:2220–2225.

10 Kawai H, Nakai H, Suga M, Yuki S, Watanabe T, Saito KI. Effects of a novel free radical scavenger, MCl-186, on ischemic brain damage in the rat distal middle cerebral artery occlusion model. J Pharmacol Exp Ther. 1997;281:921–927.

11 Abe K, Yuki S, Kogure K. Strong attenuation of ischemic and postischemic brain edema in rats by a novel free radical scavenger. Stroke. 1988;19:480–485.

12 Donnan GA, Fisher M, Macleod M, Davis SM. Stroke. Lancet. 2008;371:1612–1623.

13 Zong XM, Sun YF, Pei DS, Zeng YM, Xu T. Pergolide protects CA1 neurons from apoptosis in a gerbil model of global cerebral ischemia. Neurol Res. 2008;30:92–98.

14 Zhang Y, Deng P, Ruan Y, Xu ZC. Dopamine D1-like receptors depress excitatory synaptic transmissions in striatal neurons after transient forebrain ischemia. Stroke. 2008;39:2370–2376.

15 Narkar V, Kunduzova O, Hussain T, Cambon C, Parini A, Lokhandwala M. Dopamine D2-like receptor agonist bromocriptine protects against ischemia/reperfusion injury in rat kidney. Kidney Int. 2004;66:633–640.

16 Seeman P, Van Tol HH. Dopamine receptor pharmacology. Trends Pharmacol Sci 1994;15:264–270.

17 Khaliulin I, Schneider A, Houminer E, Borman JB, Schwalb H. Apomorphine prevents myocardial ischemia/reperfusion-induced oxidative stress in the rat heart. Free Radic Biol Med. 2004;37:969–976.

18 Khaliulin I, Schneider A, Houminer E, Borman JB, Schwalb H. Apomorphine-induced myocardial protection is due to antioxidant and not adrenergic/dopaminergic effects. Free Radic Biol Med. 2006;40:1713–1720.

19 Ishige K, Chen Q, Sagara Y, Schubert D. The activation of dopamine D4 receptors inhibits oxidative stress-induced nerve cell death. J Neurosci. 2001;21:6069–6076.

20 Okada Y, Sakai H, Kohiki E, Suga E, Yanagisawa Y, Tanaka K, et al. A dopamine D4 receptor antagonist attenuates ischemia-induced neuronal cell damage via upregulation of neuronal apoptosis inhibitory protein. J Cereb Blood Flow Metab. 2005;25:794–806.

21 Hanson ES, Leibold EA. Regulation of iron regulatory protein 1 during hypoxia and hypoxia/reoxygenation. J Biol Chem. 1998;273:7588–7593.

22 Tamatani M, Mitsuda N, Matsuzaki H, Okado H, Miyake S, Vitek MP, et al. A pathway of neuronal apoptosis induced by hypoxia/

reoxygenation: roles of nuclear factor-kappaB and Bcl-2. J Neurochem. 2000;75:683–693.

23 Kosuge Y, Koen Y, Ishige K, Minami K, Urasawa H, Saito H, et al. S-allyl-L-cysteine selectively protects cultured rat hippocampal neurons from amyloid beta-protein- and tunicamycin-induced neuronal death. Neuroscience. 2003;122:885–895.

24 Levraut J, Iwase H, Shao ZH, Vanden Hoek TL, Schumacker PT. Cell death during ischemia: relationship to mitochondrial depolarization and ROS generation. Am J Physiol Heart Circ Physiol. 2003;284:H549–H558.

25 Patel S, Patel S, Marwood R, Emms F, Marston D, Leeson PD, et al. Identification and pharmacological characterization of [125I]L-750,667, a novel radioligand for the dopamine D4 receptor. Mol Pharmacol. 1996;50:1658–1664.

26 Bowery BJ, Razzaque Z, Emms F, Patel S, Freedman S, Bristow L, et al. Antagonism of the effects of (+)-PD 128907 on midbrain dopamine neurones in rat brain slices by a selective D2 receptor antagonist L-741,626. Br J Pharmacol. 1996;119:1491–1497.

27 Sautel F, Griffon N, Sokoloff P, Schwartz JC, Launay C, Simon P, et al. Nafadotride, a potent preferential dopamine D3 receptor antagonist, activates locomotion in rodents. J Pharmacol Exp Ther. 1995;275:1239–1246.

28 Matulenko MA, Hakeem AA, Kolasa T, Nakane M, Terranova MA, Uchic ME, et al. Synthesis and functional activity of (2-aryl-1-piperazinyl)-N-(3-methylphenyl) acetamides: selective dopamine D4 receptor agonists. Bioorg Med Chem. 2004;12:3471–3483.

29 Tan S, Sagara Y, Liu Y, Maher P, Schubert D. The regulation of reactive oxygen species production during programmed cell death. J Cell Biol. 1998;141:1423–1432.

30 Tan S, Wood M, Maher P. Oxidative stress induces a form of programmed cell death with characteristics of both apoptosis and necrosis in neuronal cells. J Neurochem. 1998;71:95–105.

31 Li Y, Maher P, Schubert D. Requirement for cGMP in nerve cell death caused by glutathione depletion. J Cell Biol. 1997;139:1317–1324.

32 Ishimura A, Ishige K, Taira T, Shimba S, Ono S, Ariga H, et al. Comparative study of hydrogen peroxide- and 4-hydroxy-2-nonenal-induced cell death in HT22 cells. Neurochem Int. 2008;52:776–785.