Different Impacts of α- and β-Blockers in Neurogenic Hypertension Produced by Brainstem Lesions in Rat

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ABSTRACT

Background: Bilateral lesions of nucleus tractus solitarii in rat result in acute hypertension, pulmonary edema, and death within hours. The hypertension results from excessive catecholamine release. Catecholamine can activate connexin43 to regulate cell death. There is no study investigating the cardiopulmonary impacts of different adrenergic blockers and apoptosis mechanism in rat model.

Methods: The authors microinjected 6-hydroxydopamine into nucleus tractus solitarii of the rat (n = 8 per group) and evaluated the cardiopulmonary changes after treatment with different concentrations of α1-blockers, α2-blockers, β-blockers, and α-agonists.

Results: In the rat model, the authors found that prazosin (0.15 mg/kg) treatment could preserve cardiac output and reverse neutrophil infiltrations in lungs and lead to prevent pulmonary hemorrhagic edema. The time-dependent increases in connexin43 and terminal deoxynucleotidyl transferase dUTP nick end labeling–positive cells induced by 6-hydroxydopamine lesions were decreased after prazosin treatment (terminal deoxynucleotidyl transferase dUTP nick end labeling–positive cells at 6 h: 64.01 ± 2.41% *vs.* 24.47 ± 3.10%; mean ± SD, $P < 0.001$, in heart, and 80.83 ± 2.52% *vs.* 2.60 ± 1.03%, $P < 0.001$, in lung). However, propranolol caused further compromise of the already impaired cardiac output with consequence of rapid death. Phenylephrine enhanced the phenotype in the link between connexin43 expressions and terminal deoxynucleotidyl transferase dUTP nick end labeling–positive cells but not yohimbine. Connexin43 expressions and terminal deoxynucleotidyl transferase dUTP nick end labeling–positive cells were more decreased with prazosin (0.15 and 0.3 mg/kg) than that with prazosin (0.05 mg/kg) treatment.

Conclusions: α1-Receptors are the keystones of the phenotype. In some brainstem encephalitis and brain injury with nucleus tractus solitarii involvement, early α1-receptor blockade treatment may prevent acute death from tissue apoptosis. α-Blockers can also decrease cerebral perfusion pressure, and further studies are needed in translation to brain injury with increased intracranial pressure. **(ANESTHESIOLOGY 2014; 120:1192-204)**

THE nucleus tractus solitarii (NTS) is a primary site of termination of baroreceptors and plays an important role in the integration of inhibitory regulation in the sympathetic nervous system.[1] Bilateral lesions of the NTS result in the rapid development of acute fulminating hypertension. It is due to a marked augmentation of sympathetic nerve activity, excessive catecholamine release into the plasma, and then produces a marked increase in peripheral resistance, which leads to a reduction in cardiac output, progressive heart failure, pulmonary edema, and death.[2]

In our previous study,[2] we used the ganglionic blocker (hexamethonium) to block the acute large release of catecholamines for reducing serum levels of catecholamines to attenuate the deleterious effects on a variety of physiologic markers of cardiopulmonary injury in the rat model.

What We Already Know about This Topic

- Bilateral lesions of nucleus tractus solitarii in rat result in acute fulminating hypertension, decreased cardiac output, neurogenic pulmonary edema, and death within hours resulting from excessive catecholamine release
- The modulatory role of adrenergic blockers on cardiovascular response and apoptosis remains unknown

What This Article Tells Us That Is New

- Using a rat model of lesions of the nucleus tractus solitarii and various adrenergic antagonists, it was shown that α1-adrenoceptors are the cornerstone for this phenotype

However, hexamethonium acts on receptors at preganglionic sites in both the sympathetic and parasympathetic nervous

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systems and the adverse effects of hexamethonium combine sympatholytic and parasympatholytic effects.

The hypertension produced by bilateral NTS lesions resulted from an increased peripheral vascular resistance. This was due to intensive vasoconstriction secondary to augmented discharge of sympathetic preganglionic neurons and is primarily mediated by α-receptors.[3] Besides, the release of catecholamines, especially α-adrenergic agonists, can activate the inflammatory cascade in the lung and provide a link between brain injury and development of acute lung injury.[4,5]

Connexin43 (Cx43) is thought to be an important gap junction protein for cell-to-cell communication and cell signaling among themselves and with their environment.[6–8] Catecholamine can increase Cx43 expressions after adrenoceptor stimulation with enhanced gap junctional intercellular communication.[8] Cardiac Cx43 not only plays an essential role in cell–cell coupling and normal cardiac function but also participates in cell survival and cell death.[6] In addition, Cx43-mediated gap junctions spread proinflammatory signals in the lung capillary bed, leading to acute lung injury by inflammation that develops rapidly across the vascular surface of the lung.[9] Cellular apoptosis can mediate cell death in a variety of heart and lung diseases.[10,11]

Postganglionic sympathetic systems are usually regulated by adrenergic receptors and the adrenergic receptors are targets of the catecholamines, especially norepinephrine and epinephrine. In clinical practice, α-blocker is used in the treatment of hypertension and β-blockers have been shown to have favorable effects on left ventricle remodeling and improving clinical outcome.[12] There is no previous study to investigate the effects of adrenergic blockers and the role of apoptosis in the rat model with NTS-induced hypertension. We hypothesized that α1-blockade is effective in markedly attenuating the deleterious effects on a variety of physiologic markers of cardiopulmonary injury, inflammation, and apoptosis in NTS-induced hypertension. For investigating more specific drugs to prevent nonspecific adverse effects and the link between Cx43 expression and cell death with apoptosis mechanisms in the rat model, the aims of this study were to investigate (1) the individual effect of α- and β-blockers on pathological and functional changes of the heart and lungs; and (2) Cx43 regulating cellular apoptosis in neurogenic hypertension produced by brainstem lesions in the rat for clinical applications.

Materials and Methods

Animals and Experimental Design

Male Sprague–Dawley rats (weight, 270 to 360 g) were obtained from the National Science Council Animal Facility and housed in the animal room of the Kaohsiung Veterans General Hospital (Kaohsiung, Taiwan, R.O.C.). The rats were kept in cages in a room (lighting, 12 h on/12 h off, temperature 23° to 24°C). The rats were given normal rat chow (Ralston Purina Co., St. Louis, MO) and tap water *ad libitum* and acclimatized to the housing conditions for 1 week. The animal research protocols were approved by the Research Animal Facility Committee of the Kaohsiung Veterans General Hospital.

The rats were divided into three groups. Group 1 was a control group that received microinjections of 6-hydroxydopamine (6-OHDA, 30 μg; Sigma-Aldrich Co., St. Louis, MO) dissolved in a vehicle (0.8% ascorbic acid in saline) into the NTS bilaterally. Group 2 was an intervention group that received infusions of the α1-adrenergic blocker (prazosin, 0.15 mg/kg, i.v.; Sigma-Aldrich Co.) *via* the femoral vein at the time point of 10 min after the NTS lesion. Group 3 was another intervention group that received infusions of the β-adrenergic blocker (propranolol, 1 mg/kg, i.v.; Sigma-Aldrich Co.) *via* the femoral vein at the time point of 10 min after the NTS lesion. First, each group of rats (n = 8 in each group) was anesthetized with urethane (1 g/kg, intraperitoneal; Sigma-Aldrich Co.) and connected to oscillator circuits for blood pressure and heart rate (HR) recording *via* the femoral artery. In detail, a polyethylene cannula was inserted into the femoral artery and connected to a pressure transducer (Gould P23 ID; Gould Instruments, Cleveland, OH) and a polygraph (AT5000; Gould) to measure blood pressure directly, and a tachograph preamplifier (13-4615-65; Gould) to monitor HR continuously. Tracheotomy was performed to keep airway patent during the experiment. Second, we determined some time points for analysis. The rats were anesthetized with pentobarbital (50 mg/kg, intraperitoneal; Sigma-Aldrich Co.) before the NTS lesion with 6-OHDA. After 6-OHDA-induced NTS lesions and intervention with α1-adrenergic blocker or β-adrenergic blocker, the wounds were closed and the rats removed from the stereotaxic frame for further observation. Each rat was placed in a cage and woken within half-an-hour for free activity. The rats were sacrificed at different time points (group 1 at 0, 3, and 6 h and group 2 at 0, 3, 6, and 12 h, n = 8 for each time point) for analyzing histopathology and biochemistry of heart and lung samples. Before sacrifice, cardiac structure and function were examined by echocardiography.

To demonstrate the roles of α-receptors in the phenotype, we performed the experiments that received infusions of (1) α-agonist (phenylephrine, 20 μg/kg, i.v.; Sigma-Aldrich Co.), (2) α-2 blocker (yohimbine, 2 mg/kg, i.v.; Sigma-Aldrich Co.), and (3) different concentrations of α1-blocker (prazosin, 0.05, 0.15, and 0.3 mg/kg, i.v.) *via* the femoral vein at the time point of 10 min after the NTS lesion. We investigated Cx43 expressions and apoptotic cell death 6 h after 6-OHDA-induced NTS lesions.

Microinjections of 6-OHDA into the NTS of Rats

The rat was placed into a stereotaxic instrument (Kopf Instruments, Tujunga, CA) with the head downward at a 45° angle. We performed a craniotomy to expose the dorsal surface of the medulla and then the rat was allowed to rest for

at least 1 h before experiments. Single-barrel glass pipettes (0.031-inch OD, 0.006-inch ID; Richland Glass Co Inc., Vineland, NJ) were fixed onto the stereotaxic holder and connected to a Hamilton microsyringe through polyvinyl tubing for microinjections into the NTS.[13]

We identified the NTS as described previously,[14,15] a pipette was filled with L-glutamate (0.154 nmol/60 nl) and lowered into the NTS with anteroposterior coordinates 0.0 mm; mediolateral, 0.5 mm; and vertical, 0.4 mm with the obex. A specific decrease in blood pressure and HR (≥−35 mmHg and −50 beats/min) was demonstrated after microinjection of L-glutamate into the NTS.

Catecholamine Levels and Pulmonary Edema Index

Blood samples were collected into microtubes from the inferior vena cava and placed immediately into an ice bath. The samples were centrifuged at 10,000 rpm for 20 min at 4°C and aliquots of plasma were removed and stored at −80°C. We assayed the epinephrine and norepinephrine contents using an enzyme-linked immunoassay (Labor Diagnostika Nord, GmbH & Co., KG, Nordhorn, Germany).

After taking the blood samples, rats were sacrificed and the chest was opened to remove the lungs and heart. Each set of lungs was weighed to estimate liquid accumulation in the lungs, then dried in an oven at 80°C for 2 days, and reweighed to determine dry lung weight. The wet-to-dry lung weight ratio was calculated to indicate the degree of pulmonary edema.[2]

Histology and Immunohistochemical Analysis

As described previously,[2] the rat heart and lungs were excised immediately after sacrifice for hematoxylin and eosin staining. The specimens were placed in 10% formalin for 5 days, then blocked, embedded in paraffin, cut into 4 μm sections, and stained with hematoxylin and eosin.

In immunohistochemical analysis,[2] the heart and lung tissues were performed on the sections after the paraffin was melted in an oven at 70°C for 1 h for assay of troponin T and brain natriuretic peptide in heart, neutrophil cells in lung, and Cx43, caspase3, and apoptotic cells in heart and lung. The sections were deparaffinized, microwaved in citric buffer (10 mmol/l, pH 6.0), quenched in 30% H_2O_2–methanol, blocked in 3% goat serum, and incubated with anticardiac troponin T antibody (1:200; Abcam plc., Cambridge, England), antibrain natriuretic peptide polyclonal antibody (1:1,000; Thermo Fisher Scientific Inc., Waltham, MA), antineutrophil elastase antibody (1:400; Abcam plc.), anti-Cx43/GJA1 antibody (1:200 in heart and 1:400 in lung; Abcam plc.), antiactive caspase 3 antibody (1:200 in heart and 1:250 in lung; Abcam plc.), and anti-BrdU antibody (TACS-XL *In Situ* Apoptosis Detection Kit; Trevigen, Gaithersburg, MD) overnight at 4°C. Next, the sections were incubated with biotinylated secondary antibodies (1:200; Vector Laboratories, Burlingame, CA) for 1 h and in AB complex (1:100; Vector Laboratories) for 30 min at room temperature. The sections were visualized using a DAB substrate kit (Vector Laboratories) and counterstained with hematoxylin. The sections were then photographed using a microscope mounted with a charge-coupled device camera.

Echocardiography

As described previously,[2,16] we performed echocardiographic examinations using commercially available equipment (Vivid 7; GE Vingmed Ultrasound AS, Horten, Norway) equipped with 10-MHz-phased array transducers. The recordings were stored digitally as two-dimensional cine loops and analyzed with software (Echo PAC; GE Vingmed Ultrasound AS).

Statistical Analysis

The sample size calculation was based on the expected effect size which was provided in our previous study[2] and calculated from G*Power version 3.1.7. (Franz Faul, Universitat Kiel, Germany). A power analysis was performed using the α level of 0.05, 1-β of 0.80, variance explained special effect of 8.742, error variance of 8.542, and effect size of 1.01 which was gotten from the Cx43 data of our previous study.[2] An *a priori* test computed the required sample size for performing *F* tests ANOVA: fixed effects, special, main effects, and interactions. The minimum required sample sizes to find a statistically significant difference among 0, 3, and 6 h between control and treatment groups were calculated. We estimated that a minimum number of total sample sizes n = 15 would be required.

Group data were expressed as means ± SD. The software SPSS Statistics 17.0 (SPSS Inc., Chicago, IL) was used to conduct the statistical analyses. A two-way repeated-measures ANOVA was used to evaluate the blood pressure and HR with treatment effect, time effect, and check for interaction (with Bonferroni *post hoc* test within the same group). A two-way ANOVA was conducted with treatment effect, time effect, and check for interaction (with Tukey *post hoc* test) to analyze parameters of brain natriuretic peptide, troponin T, wet-to-dry lung weight ratio, neutrophils, Cx43 (heart and lung), caspase3 (heart and lung), terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)–positive cells (heart and lung), left ventricle internal dimensions in diastole and systole, cardiac output, interventricular septum thickness in diastole, and left ventricle posterior wall thickness in diastole. We found that significant effects are present in assessing time effect (0, 3, and 6 h), treatment effect (6-OHDA *vs.* 6-OHDA plus prazosin), and the interactions between the time and treatment for all the tests except in the time effect of cardiac output. In addition, Student *t* test was applied to compare two groups at the same sacrificed time point (0, 3, and 6 h in group 1 *vs.* group 2; 6 h in group 1 *vs.* phenylephrine or yohimbine). A *P* value of less than 0.05 (two-tailed) was used to establish statistical significance.

Results

Rats with 6-OHDA lesions of the NTS (fig. 1A) exhibited labored breathing and grunting respiration beginning at 2 to 3 h post-NTS lesion and died within 7 h (fig. 1B). Some pink frothy fluid was found in the rats' nostrils before death. When we administered prazosin (0.15 mg/kg) post-6-OHDA NTS lesion, all the rats survived for more than 14 h (fig. 1C) and did not exhibit labored breathing. In the blood pressure recordings (fig. 1D), an acute increase in blood pressure induced by the 6-OHDA NTS lesions was reversed immediately to below the baseline level by treatment with prazosin. In the HR recordings (fig. 1E), we found significantly increased HR at 12 h in the prazosin-treated group. In the epinephrine (fig. 1F) and norepinephrine (fig. 1G) serum levels, we found that the serum levels of epinephrine and norepinephrine increased largely and acutely within 3 h and remained high until 6 h in both the 6-OHDA and 6-OHDA plus prazosin groups. There was no significant difference between these two groups within 6 h.

In the histological changes observed in myocardial fibers in longitudinal sections, the appearance of irregular wavy fibers in hematoxylin- and eosin-stained preparations, increased eosinophilic staining of the cytoplasm and contraction band necrosis reflecting the hypercontracted state of the cells at 3 and 6 h in the 6-OHDA group were prevented by the prazosin treatment. In the immunohistochemical staining for brain natriuretic peptide (fig. 2A) and cardiac troponin T (fig. 2B), the increased strong expressions of brain natriuretic peptide and troponin T at 3 h and additionally increased expressions at 6 h caused by the 6-OHDA lesions were significantly attenuated by the prazosin treatment.

In the gross specimens of the lungs (fig. 2C), it was observed that severe pulmonary hemorrhagic edema developed at 6-h post-6-OHDA NTS lesion, but no pulmonary edema was found within 12 h with the prazosin treatment. In hematoxylin- and eosin-stained preparations, the appearances of increased cell infiltrations and erythrocytes in the lung interstitium at 3 h and hyaline membrane formation at 6 h in the 6-OHDA group were attenuated or prevented by the prazosin treatment (fig. 2D). The increased infiltrating cells were found to be neutrophils by immunohistochemical staining (fig. 2E).

In analyses of Cx43- and caspase3-expressing and TUNEL-positive cells in the heart (fig. 3) and lungs (fig. 4), we found that the time-dependent increased and enhanced expression of Cx43, caspase3, and the TUNEL-positive cells induced by the 6-OHDA lesion were decreased within 3 and 6 h after the prazosin treatment.

In echocardiographic M-mode images of the parasternal short-axis view (fig. 5, A and B), we found that the left ventricle internal dimensions in diastole were significantly decreased at 3 h and more decreased at 6 h in the 6-OHDA group but were preserved with no significant changes over 12 h in the 6-OHDA plus prazosin group (fig. 5C). Although there were significantly decreased left ventricle internal dimensions in systole at 3 and 6 h in both groups (6-OHDA and 6-OHDA plus prazosin), these dimensions were reversed at 6 h and preserved at 12 h in the 6-OHDA plus prazosin group (fig. 5D). The cardiac output calculated according to the above two parameters showed decreased cardiac output at 3 h and more decreased output at 6 h in the 6-OHDA group which was preserved in the 6-OHDA plus prazosin group (fig. 5E). The increased left ventricle wall thickness induced by the 6-OHDA lesions due to enhanced contractility and impaired diastolic function[2] after massive catecholamine release at 3 and 6 h was reversed by the prazosin treatment (fig. 5, F and G).

In rats with bilateral 6-OHDA lesions of the NTS and treatment with the β-receptor blocker propranolol, we found that all the rats died within 2 h (fig. 6A). The acute hypertension induced by the 6-OHDA lesion was acutely reversed toward normality, accompanied by a significant decrease in HR after propranolol treatment (fig. 6B). In autopsy, the rats showed acute dilation of the left ventricle (fig. 6C), the appearance of some irregular wavy fibers in hematoxylin- and eosin-stained preparations, and increased eosinophilic staining of cytoplasm in the heart (fig. 6C). In the autopsy of the lungs, some hemorrhagic spots were observed in gross (fig. 6D) and erythrocyte infiltration in hematoxylin- and eosin- stained preparations (fig. 6D). At death time, as compared with 0 h, the ratio of wet-to-dry lung weights and immunohistochemical analyses of neutrophils in the lungs did not change significantly, and no pulmonary edema was noted. There were also no significant changes in the immunohistochemical analyses of brain natriuretic peptide, troponin T, Cx43, caspase3, and TUNEL-positive cells in the heart, and Cx43, caspase3, and TUNEL-positive cells in the lungs.

Testing α-receptors are the keystones of the phenotype, at 6 h after 6-OHDA-induced NTS lesions, we found that α-agonist (phenylephrine) increased the Cx43 expressions and TUNEL-positive cells of the heart and lungs (fig. 7A) and α-2 blocker (yohimbine) did not change the Cx43 expressions and TUNEL-positive cells of the heart and lungs (fig. 7B). The Cx43 expressions and TUNEL-positive cells of the heart and lungs were decreased significantly with prazosin (0.05 mg/kg) treatment and further decreased with prazosin (0.15 and 0.3 mg/kg) treatment (fig. 7C).

Discussion

In the rat model of 6-OHDA-induced NTS lesions, we found that prazosin could prevent cardiac hypercontractility and preserve cardiac output observed by echocardiography. It also reversed the pathological changes of contraction band necrosis and increased expressions of brain natriuretic peptide and troponin T in the heart and neutrophil infiltration in the lungs, leading to prevention of hyaline membrane formation and pulmonary hemorrhagic edema, increasing survival time. In contrast, propranolol caused further compromise of the already impaired cardiac output with

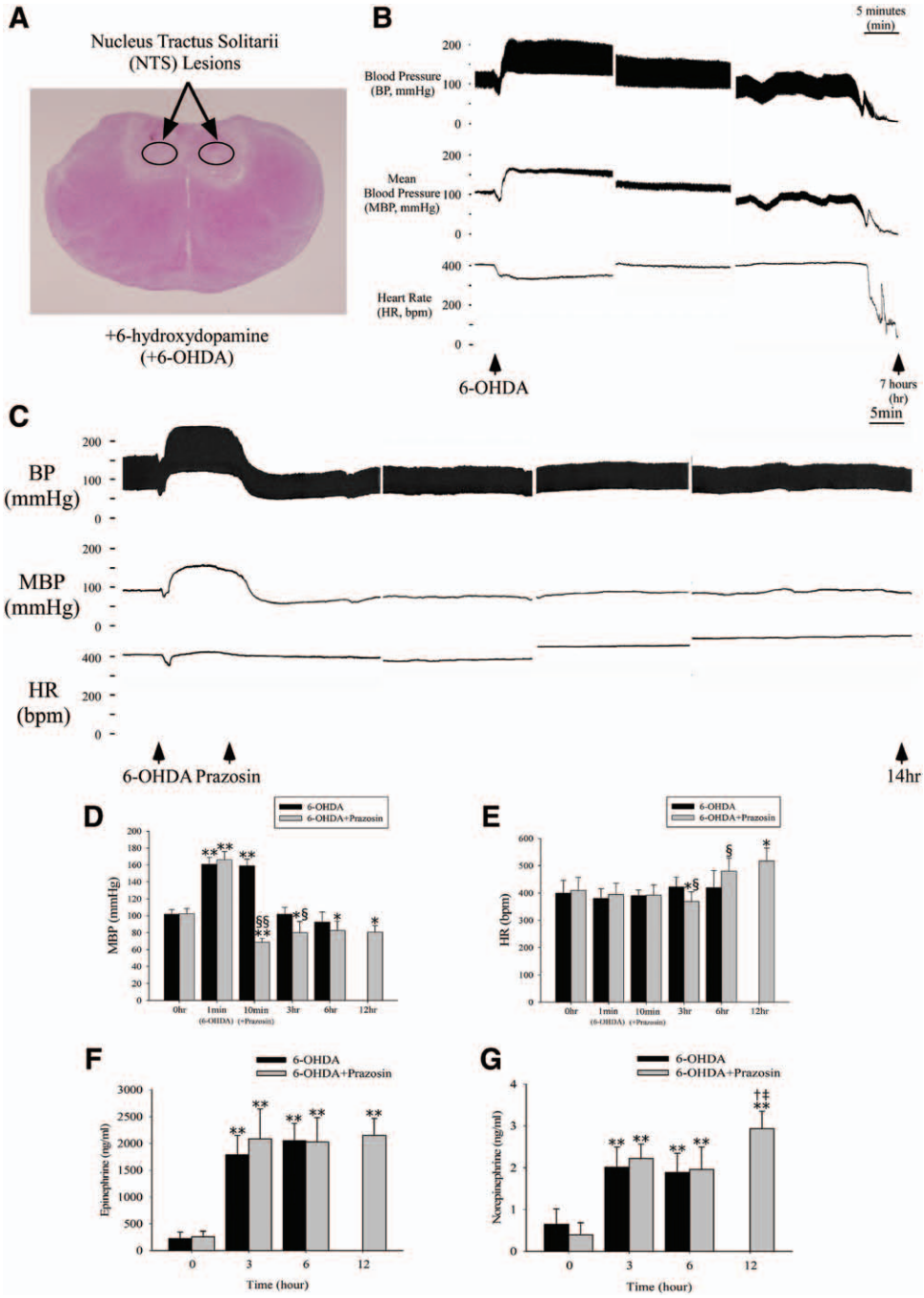

Fig. 1. Treatment with prazosin (0.15 mg/kg) reversed 6-hydroxydopamine (6-OHDA)-induced acute hypertension and increased survival time. (*A*) Representative nucleus tractus solitarii (NTS) position of 6-OHDA lesions (*arrows*). (*B*) Rats with bilateral 6-OHDA lesions of NTS died suddenly within 7 h with severe pulmonary hemorrhagic edema complications developing before death (n = 8). (*C*) Rats with bilateral 6-OHDA lesions of NTS survived for at least 14 h with no pulmonary edema at 14 h when treated with prazosin (n = 8). (*D*) Rats with 6-OHDA lesions had a significantly high acute increase in their mean blood pressure. The acute increase in mean blood pressure was acutely reversed to below baseline levels when prazosin was administered 10 min after the 6-OHDA lesions were induced (n = 8). (*E*) Heart rate increased significantly after 6 h in the group of rats treated with prazosin (n = 8). (*F*) An acute and excessive increase in the epinephrine serum level was induced within 3 h and remained high until 6 h post-6-OHDA NTS lesion. The change in the epinephrine serum level was similar in the intervention with the prazosin treatment and remained high until 12 h (n = 8 per group). (*G*) An acute and excessive increase in the norepinephrine serum level was induced within 3 h and remained high until 6 h post-6-OHDA NTS lesions. The change in the norepinephrine serum level was similar within 6 h in the intervention with the prazosin treatment and higher at 12 h (n = 8 per group). The data represent the means ± SD. **P* < 0.05 and ***P* < 0.001 *versus* the respective group at 0 h. †*P* < 0.05 *versus* the respective group at 3 h. ‡*P* < 0.001 *versus* the respective group at 6 h. §*P* < 0.05 and §§*P* < 0.001 6-OHDA *versus* 6-OHDA plus prazosin.

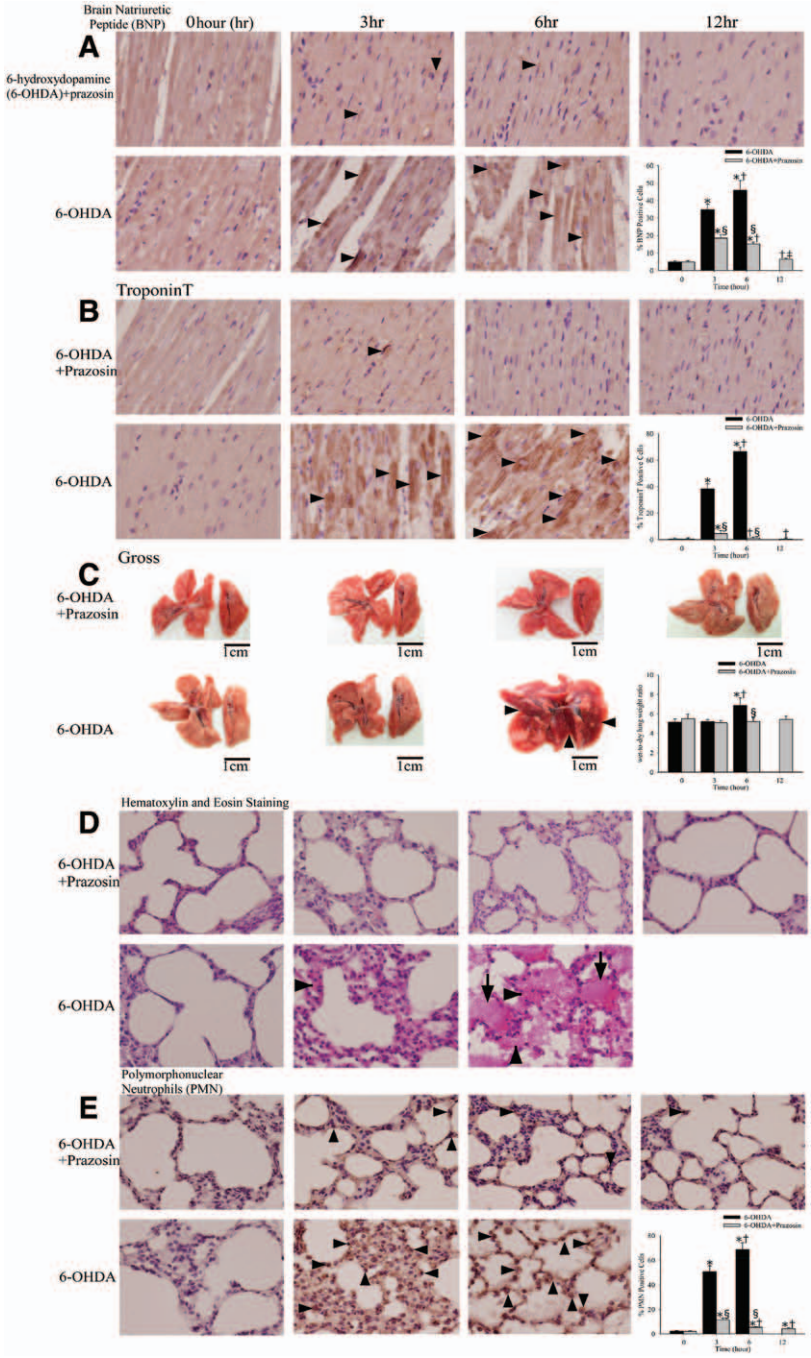

Fig. 2. Treatment with prazosin (0.15 mg/kg) reversed 6-hydroxydopamine (6-OHDA)-induced pathological changes in the heart and lung (magnification ×400). (*A*) Representative examples of the immunocytochemistry of brain natriuretic peptide in rat heart. The increased brain natriuretic peptide staining (*arrowheads*) in the cytoplasm at 3 and 6 h in the 6-OHDA group was reduced by prazosin treatment (n = 8 per group). (*B*) Representative examples of immunocytochemistry of cardiac troponin T in rat heart. The increased troponin T staining (*arrowheads*) in the cytoplasm at 3 and 6 h in the 6-OHDA group was reduced by prazosin treatment (n = 8 per group). (*C*) Representative examples of the rat lung. Treatment with prazosin prevented the mild and severe lung congestion (*arrowheads*) induced in the rats with 6-OHDA lesions at 3 and 6 h (n = 8 per group). The wet-to-dry lung weight ratio was increased at 6 h in the 6-OHDA group and reversed by prazosin treatment (n = 8 per group). (*D*) Representative examples of hematoxylin- and eosin-stained lung specimens. Increased infiltration of erythrocytes (*arrowheads*) at 3 h and formation of a hyaline membrane (*arrows*) at 6 h in the 6-OHDA group were attenuated by prazosin treatment (n = 8 per group). (*E*) Representative examples of the immunocytochemistry of polymorphonuclear neutrophils (*arrowheads*) in rat lung sections. The increased infiltrations of polymorphonuclear neutrophils at 3 and 6 h in the 6-OHDA group were reduced by prazosin treatment (n = 8 per group). The data represent the means ± SD. **P* < 0.001 *versus* the respective group at 0 h; †*P* < 0.001 *versus* the respective group at 3 h; ‡*P* < 0.001 *versus* the respective group at 6 h; §*P* < 0.001 6-OHDA *versus* 6-OHDA plus prazosin.

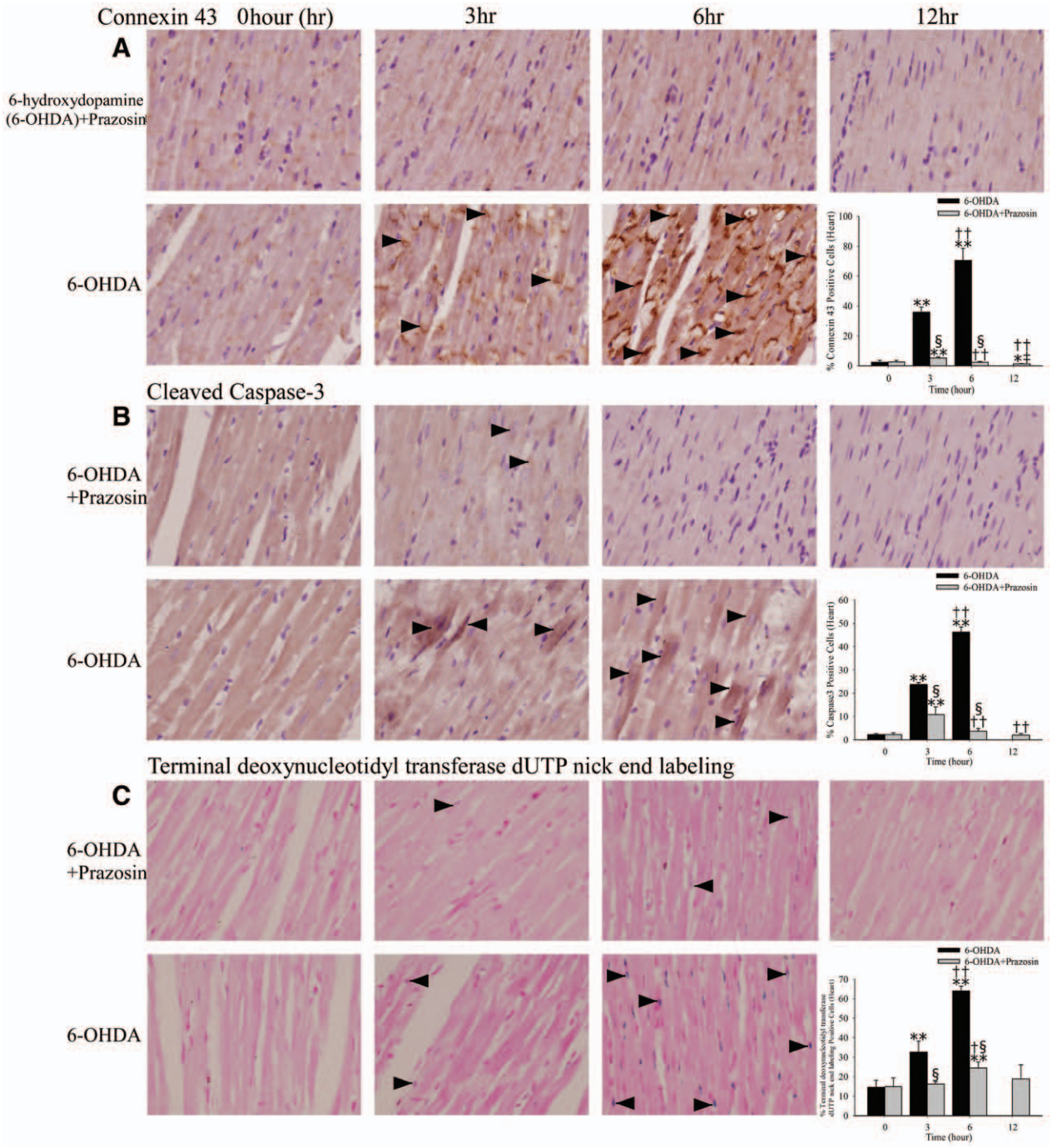

Fig. 3. Treatment with prazosin (0.15 mg/kg) inhibited the 6-hydroxydopamine (6-OHDA)-induced acute apoptotic pathway in the heart (magnification ×400). (*A*) Representative examples of immunocytochemical detection of connexin43 in rat heart muscle sections. The increased expression of connexin43 in intercalated discs (*arrowheads*) at 3 h and intense expression at 6 h in the 6-OHDA group were reduced by prazosin treatment (n = 8 per group). (*B*) Representative examples of immunocytochemical detection of caspase3 in rat heart muscle sections. The increased and strong expression levels of caspase3 (*arrowheads*) at 3 and 6 h in the 6-OHDA group were reduced by prazosin treatment (n = 8 per group). (*C*) Representative examples of *in situ* terminal deoxynucleotidyl transferase dUTP nick end labeling–positive cells detection in blue (*arrowheads*) in rat heart muscle sections. The increased and strong expression levels of terminal deoxynucleotidyl transferase dUTP nick end labeling–positive cells at 3 and 6 h in the 6-OHDA group were reduced by prazosin treatment (n = 8 per group). The data represent the means ± SD. **P* < 0.05 and ***P* < 0.001 *versus* the respective group at 0 h; †*P* < 0.05 and ††*P* < 0.001 *versus* the respective group at 3 h; ‡*P* < 0.05 *versus* the respective group at 6 h; §*P* < 0.001 6-OHDA *versus* 6-OHDA plus prazosin.

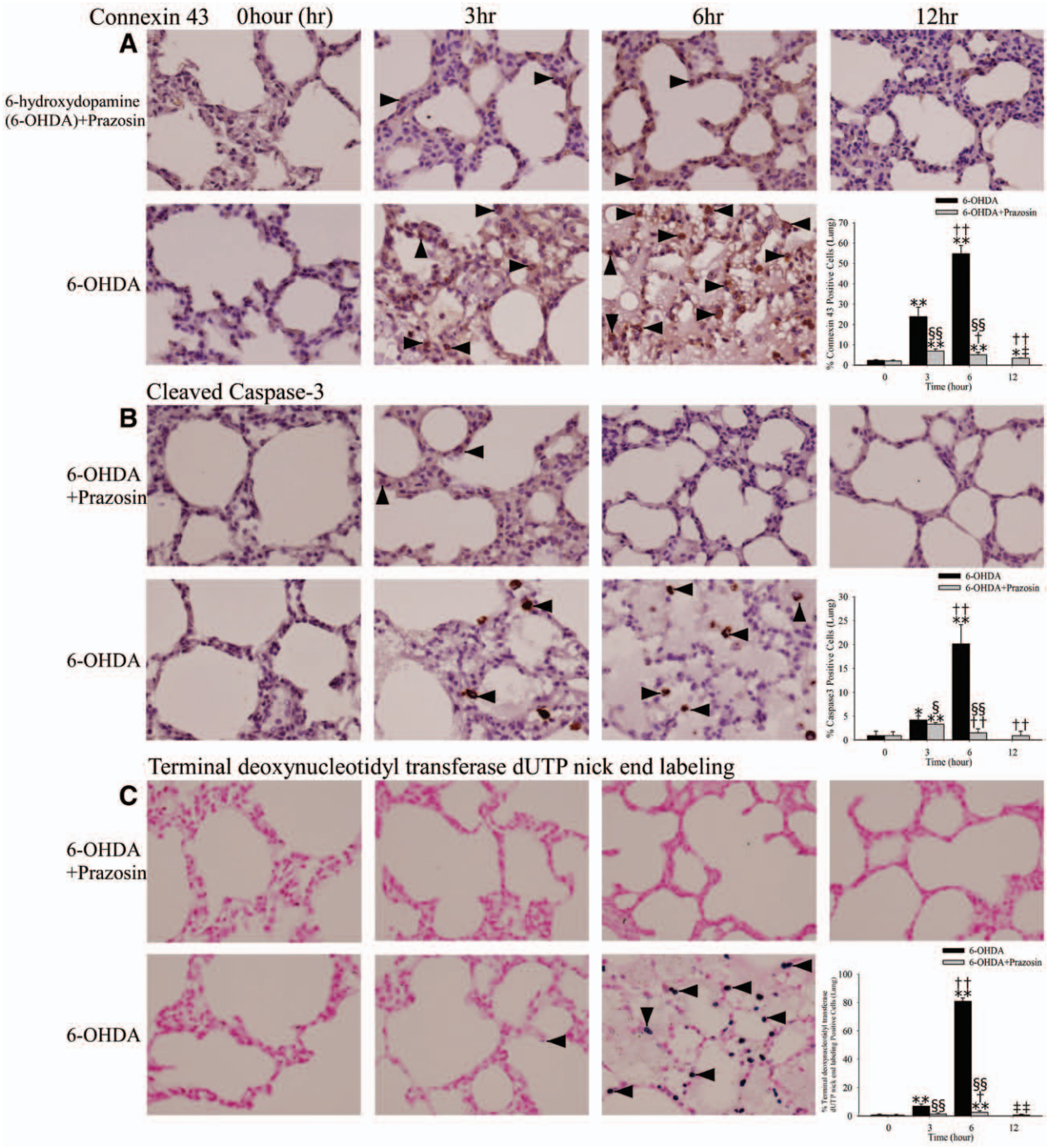

Fig. 4. Treatment with prazosin (0.15 mg/kg) inhibited the 6-hydroxydopamine (6-OHDA)-induced acute apoptotic pathway in the lung (magnification ×400). (*A*) Representative examples of immunocytochemical detection of connexin43 in rat lung sections. The increased and strong expression of connexin43 in lung (*arrowheads*) at 3 h and stronger expression at 6 h in the 6-OHDA group were reduced by prazosin treatment (n = 8 per group). (*B*) Representative examples of immunocytochemical detection of caspase3 in rat lung sections. The increased and strong expression of caspase3 (*arrowheads*) at 6 h in the 6-OHDA group was reduced by prazosin treatment (n = 8 per group). (*C*) Representative examples of *in situ* terminal deoxynucleotidyl transferase dUTP nick end labeling–positive cells detection in *blue* (*arrowheads*) in rat lung sections. The increased expression of terminal deoxynucleotidyl transferase dUTP nick end labeling–positive cells at 3 h and stronger expression at 6 h in the 6-OHDA group were reduced by prazosin treatment (n = 8 per group). The data represent the means ± SD. **P* < 0.05 and ***P* < 0.001 *versus* the respective group at 0 h; †*P* < 0.05 and ††*P* < 0.001 *versus* the respective group at 3 h; ‡*P* < 0.05 and ‡‡*P* < 0.001 *versus* the respective group at 6 h; §*P* < 0.05 and §§*P* < 0.001 6-OHDA *versus* 6-OHDA plus prazosin.

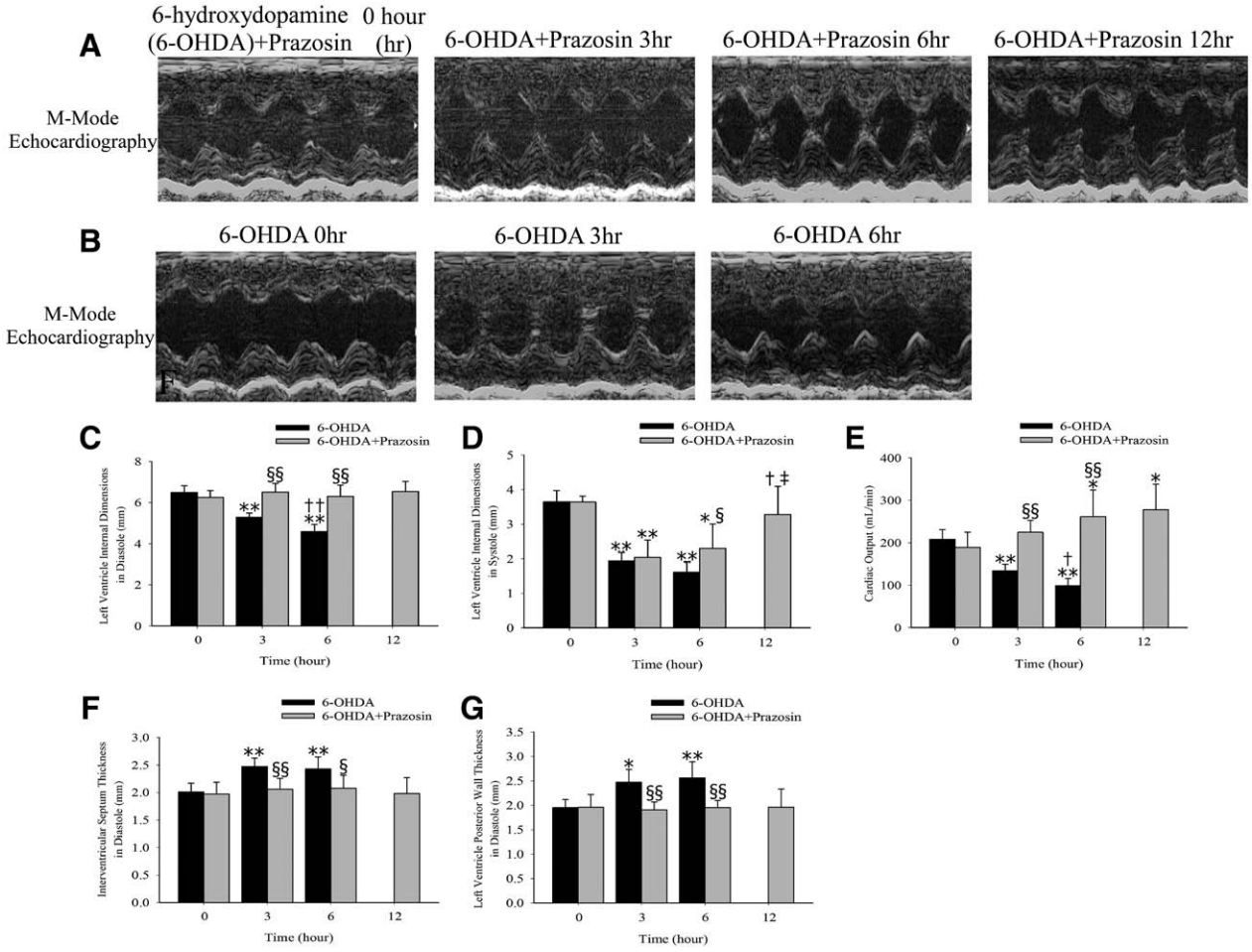

Fig. 5. Treatment with prazosin (0.15 mg/kg) preserved left ventricle internal dimensions in diastole and cardiac output (n = 8 per group). (*A* and *B*) Echocardiographic assessments of the rat by M-mode in the 6-hydroxydopamine (6-OHDA) group and with prazosin treatment (n = 8 per group). (*C*) The decrease in left ventricle internal dimensions in diastole was reversed at 3 and 6 h in the 6-OHDA plus prazosin group compared with that in the 6-OHDA group. (*D*) The left ventricle internal dimensions in systole were decreased at 3 and 6 h in the 6-OHDA group but were reversed at 6 h and increased to baseline at 12 h after prazosin treatment. (*E*) The decreased cardiac output at 3 and 6 h in the 6-OHDA group was preserved with prazosin treatment with higher cardiac output at 6 and 12 h (perhaps due to the increased heart rate). (*F* and *G*) The acute increase of the interventricular septum thickness in diastole and left ventricle posterior wall thickness in diastole were reversed at 3 and 6 h in the 6-OHDA plus prazosin group compared with that in the 6-OHDA group. The data represent the means ± SD. **P* < 0.05 and ***P* < 0.001 *versus* the respective group at 0 h; †*P* < 0.05 and ††*P* < 0.001 *versus* the respective group at 3 h; ‡*P* < 0.05 *versus* the respective group at 6 h; §*P* < 0.05 and §§*P* < 0.001 6-OHDA *versus* 6-OHDA plus prazosin.

the consequence of rapid death within 2 h. In the setting of the rat model, Cx43 is involved in an apoptotic mechanism for speeding cellular apoptosis of the heart and lungs within hours. α1-Receptors are the keystones of the phenotype. Phenylephrine can enhance the phenotype in the link between Cx43 expressions and apoptotic cell death.

Compared with our previous study,[2] the role of ganglionic blocker treatment in this model did lower catecholamine release, preserve cardiac output, and prevent pulmonary edema. The mechanism of the ganglionic blocker treatment involves the so-called "decatecholamine preconditioning" in which lowered levels of catecholamines for a short time in the beginning stages will prevent further cardiopulmonary damage from subsequent higher levels of catecholamine insults.[17] The adrenergic receptors are targets of the catecholamines, especially epinephrine and norepinephrine. In this study, we did not lower the serum levels of epinephrine and norepinephrine, but we evaluated the individual effects and different mechanisms of α- and β-adrenergic blockades in this rat model. We found that prazosin (α-blocker) alone would be beneficial for increasing survival by reducing increased vascular resistance[18] to preserve cardiac output, but propranolol (β-blocker) alone would be harmful by causing acutely increased left ventricle load[19] and acute dilatation of the left ventricle. However, the extent of pathological damage in the heart and lungs was not serious over a short time interval (<2 h of death) in the group with the propranolol treatment. Propranolol cannot

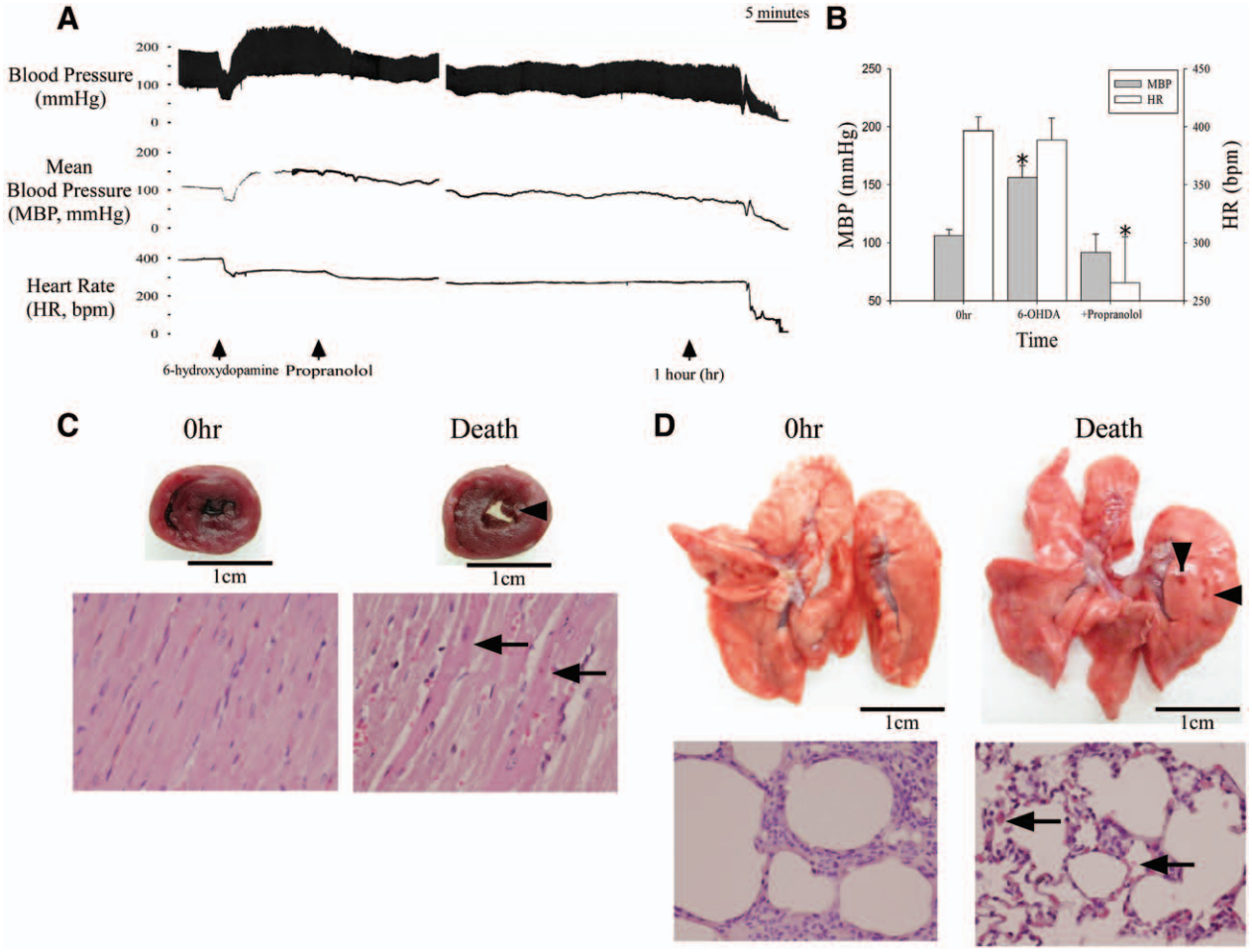

Fig. 6. Treatment with propranolol in rats with nucleus tractus solitarii lesions. (*A*) Rats with bilateral 6-hydroxydopamine (6-OHDA) lesions of the nucleus tractus solitarii died suddenly within 2 h when treated with propranolol (n = 8). (*B*) Rats with 6-OHDA lesions produced a significantly high acute increase in mean blood pressure and the acute increase in mean blood pressure was reversed acutely with decreased heart rates when propranolol was administered 10 min after the 6-OHDA lesions were induced (n = 8). (*C*) Representative examples of gross and hematoxylin- and eosin-stained morphologies in the heart. Dilatation of the left ventricle cavity (*arrowheads*) and an increase in the number of irregular wavy fibers with increased eosinophilic staining of the cytoplasm (*arrows*) when treated with propranolol post-6-OHDA lesions are shown (n = 8). (*D*) Representative examples of gross and hematoxylin- and eosin-stained morphologies in the lung. Increased hemorrhagic spots (*arrowheads*) and infiltration of erythrocytes (*arrows*) in the lung interstitium when treated with propranolol post-6-OHDA lesions are shown (n = 8). Data represent means ± SD. **P* < 0.001 *versus* 0 h.

be used alone early in the rat model with NTS lesions, but it may be useful to administer it later due to the increased HR between 6 and 12 h after prazosin treatment. In other words, the isolated usage of a β-blocker would be harmful in these settings, but we hypothesized that a β-blocker might be of benefit at a later time in the injury-recovery process. This finding may relate to other clinical process. B-blockers are commonly used to assist myocardial remodeling outside of the acute phase of illness.

Gap junctions, most importantly Cx43, are specialized structures in the plasma membranes of cells used for cell–cell coupling and cell signaling[20] and participate in decision making on cell survival or cell death.[6] In the heart, they facilitate ionic fluxes between adjacent cardiomyocytes to allow intercellular electrical communication for normal contractile function[20] and are also involved in cardioprotection by ischemic preconditioning.[21] In the lung, endothelial cells communicate Ca^{2+} signals through Cx43 for the spread of proinflammatory signals to increase microvascular permeability and cause extensive inflammation in the lung capillary bed.[9] Apoptosis is known to be programmed cell death. The novel approach to speed the cell death pathway by induction of Cx43 expression illustrated in this article may offer an important insight into apoptotic signaling in heart and lung diseases.

Isolated severe traumatic brain injury was reported to develop acute lung injury which was associated with a higher mortality and worse neurologic outcome in patients.[5] The most important cause of acute lung injury is neurogenic pulmonary edema, which is caused by

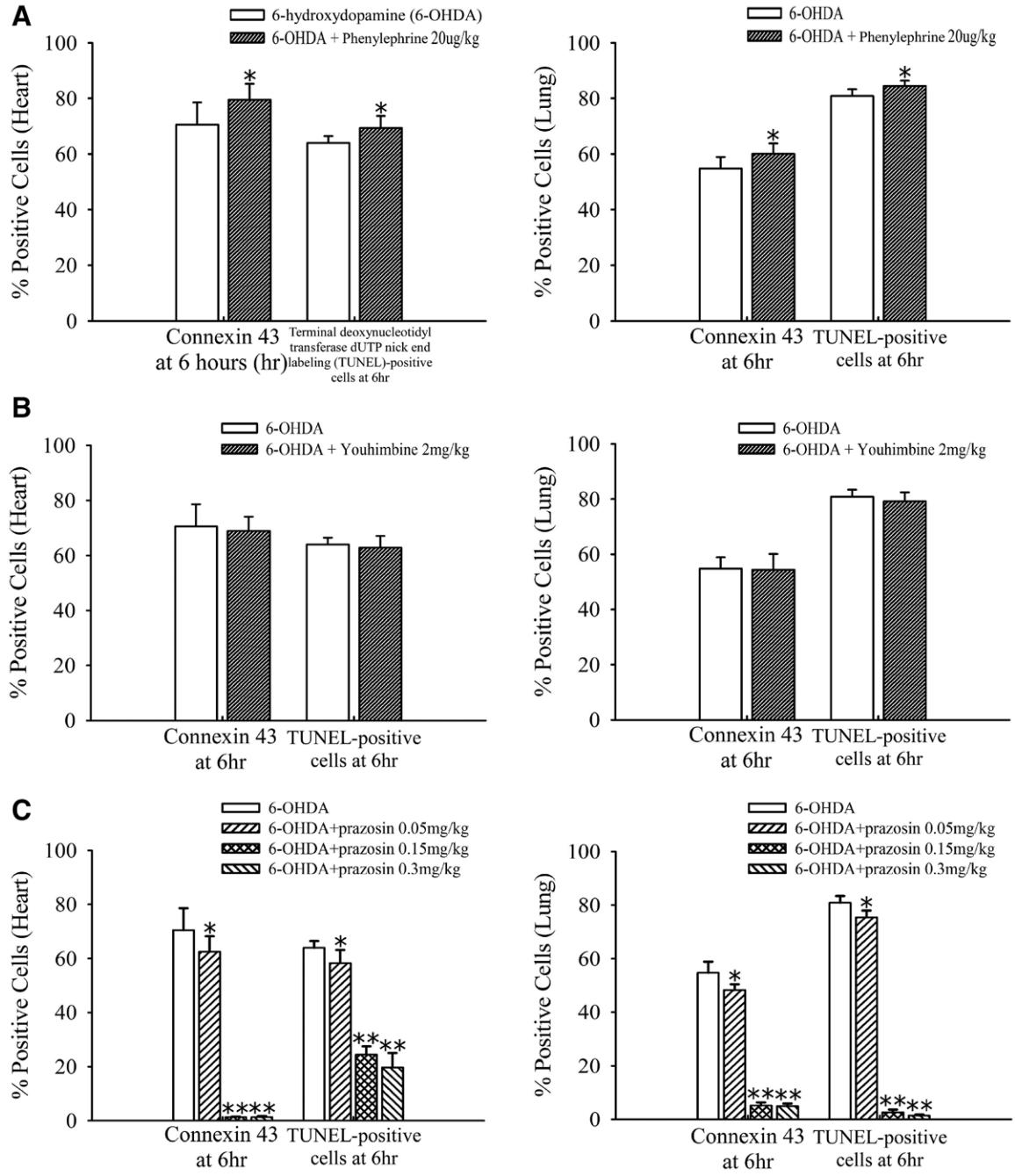

Fig. 7. The connexin43 expressions and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)–positive cells when treatment with α-agonist (phenylephrine), α-2 blocker (yohimbine), and the different concentrations of α1-blocker (prazosin) in rats with nucleus tractus solitarii (NTS) lesions. (*A*) α-Agonists treatment enhanced the connexin43 expressions and TUNEL-positive cells of the heart and lung 6 h after 6-hydroxydopamine (6-OHDA)-induced NTS lesions. (*B*) α2 Blockers treatment did not change connexin43 expressions and TUNEL-positive cells of the heart and lung 6 h after 6-OHDA-induced NTS lesions. (*C*) There were significant decreases in the connexin43 expressions and TUNEL-positive cells of the heart and lung 6 h after 6-OHDA-induced NTS lesions among 6-OHDA *versus* 6-OHDA plus prazosin 0.05 mg/kg ($P < 0.05$), 6-OHDA *versus* 6-OHDA plus prazosin 0.15 mg/kg ($P < 0.001$), and 6-OHDA *versus* 6-OHDA plus prazosin 0.3 mg/kg ($P < 0.001$). The data represent the means ± SD. *$P < 0.05$ and **$P < 0.001$ *versus* the respective group 6-OHDA.

the release of catecholamines after injury to the brainstem.[5,22–25] Certainly, the release of catecholamines, especially α-adrenergic agonists, can activate the inflammatory cascade in the lung and provide a link between severe head injury and development of acute lung injury.[4,5] Our rat model is similar to the above mechanisms and it seems reasonable to use α-adrenergic blockade to prevent acute neurogenic pulmonary edema.

Many mechanisms have been implicated in the development of neurogenic pulmonary edema, but the exact interactions among these mechanisms remain unknown.[26] Patients with various insults to the central nervous system may be complicated with acute cardiopulmonary dysfunctions resulting in neurogenic pulmonary edema and takotsubo-like cardiomyopathy. The etiology is also thought to be a massive sympathetic discharge. However, patients usually recover quickly and have a good prognosis if the patient can survive the precipitating neurologic insult.[26–28]

Takotsubo cardiomyopathy or stress cardiomyopathy is characterized by acute stress and sympathetic hyperactivity leading to clinical presentations of dyspnea, ventricular dysfunction in previously healthy people (normal heart) with no coronary artery disease.[29] In echocardiography, these patients have regional hypokinesia involving the apical and midventricular myocardial hypocontractility but preserved or enhanced basal contractility.[30] The management of stress cardiomyopathy is supportive and a complete recovery can be expected within 2 weeks. In a rat model of takotsubo cardiomyopathy,[31] the authors used rapid high-dose intravenous epinephrine bolus to mimic clinical findings during acute stress (whereas the equivalent norepinephrine bolus did not). This implied that the mechanism of takotsubo cardiomyopathy is epinephrine specific. In contrast to our rat model with NTS lesions (acute high release of both epinephrine and norepinephrine for more than 6 h), we observed acute fulminating hypertension, the intense hypercontractility of left ventricle, decreased cardiac output, progressive heart failure, neurogenic pulmonary edema, and rapid death within 7 h. The management of neurogenic hypertension after NTS-induced injury is an early intervention of α1-blockade but not β-blockade to markedly attenuate the deleterious effects on a variety of physiologic markers of cardiopulmonary injury, inflammation, and apoptosis to increase at least double the survival time.

In brainstem encephalitis[2,32] and brain injury[5,22–25] with NTS involvement, the overactivation of the sympathetic vasomotor mechanism at the medulla, resulting in acute peripheral vasoconstriction, marked increase of peripheral resistance, and a fulminant increase in arterial blood pressure induces a massive shift of blood to the low-resistance system of the pulmonary circulation. In the heart, these hemodynamic changes cause severe heart strain, induce hypercontracture in cardiac myocytes, and lead to sudden heart failure. In the lung, the accumulation of a large amount of blood in pulmonary circulation results in pulmonary hemorrhagic edema. A similar presentation can also be induced with rapid intravenous injection of a large dose of norepinephrine.[17] Our results showing the apoptotic changes in the heart and lungs of this model are compatible with *in situ* TUNEL assays of heart specimens from the patients with enterovirus rhombencephalitis which are stained for visualization of apoptotic cells.[32]

The current study may provide and add new key messages in dealing with cardiopulmonary disorders in the field of neurocritical intensive care. In this scenario of brainstem lesions, early use of α1-adrenergic blockade, not β-blockers, can decrease the hemodynamic consequences. Determination of the suitable time point for infusing the β-blocker will require further study.

In the limitations of the study, using α-blockers can prevent this phenotype; however, this treatment can also decrease cerebral perfusion pressure. In acute brain injuries with link to neurogenic hypertension *via* intracranial pressure increase, further studies with long-term behavioral tests and specific traumatic brain injury models with increased intracranial pressure are needed in translation to human acute brain injury.

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Competing Interests

The authors declare no competing interests.

Correspondence

Address correspondence to Dr. Tseng: Department of Medical Education and Research, Kaohsiung Veterans General Hospital, 386, Ta-Chung 1st Road, Kaohsiung 81362, Taiwan, R.O.C. cjtseng@vghks.gov.tw. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. Anesthesiology's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

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