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ORIGINAL INVESTIGATION

# Changes in gap junction proteins Connexin30.2 and Connexin40 expression in the sinoatrial node of rats with dexmedetomidine-induced sinus bradycardia

**Yong-Qiang Yin**[a], **Yi Zhong**[b,*], **Yu Zhu**[a], **Lei Tian**[a]

[a] *Guizhou Medical University, Guiyang, China*
[b] *Affiliated Hospital of Guizhou Medical University, Department of Anesthesiology, Guiyang, China*

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**KEYWORDS**
Autonomic nerve system;
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**Abstract**
*Background:* Dexmedetomidine (Dex) is widely used, and its most common side effect is bradycardia. The complete mechanism through which Dex induces bradycardia has not been elucidated. This research investigates the expression of gap junction proteins Connexin30.2 (Cx30.2) and Connexin40 (Cx40) within the sinoatrial node of rats with Dex-induced sinus bradycardia.
*Methods:* Eighty rats were randomly assigned to five groups. Saline was administered to rats in Group C. In the other four groups, the rats were administered Dex to induce bradycardia. In groups $D_1$ and $D_2$, the rats were administered Dex at a loading dose of 30 $\mu g.kg^{-1}$ and 100 $\mu g.kg^{-1}$ for 10 min, then at 15 $\mu g.kg^{-1}.h^{-1}$ and 50 $\mu g.kg^{-1}.h^{-1}$ for 120 min separately. The rats in group $D_1A$ and $D_2A$ were administered Dex in the same way as in group $D_1$ and $D_2$; however, immediately after the administration of the loading dose, 0.5 mg atropine was administered intravenously, and then at 0.5 $mg.kg^{-1}.h^{-1}$ for 120 min. The sinoatrial node was acquired after intravenous infusion was completed. Quantitative real-time polymerase chain reaction and western blot analyses were performed to measure mRNA and protein expression of Cx30.2 and Cx40, respectively.
*Results:* The expression of Cx30.2 increased, whereas the expression of Cx40 decreased within the sinoatrial node of rats with Dex-induced sinus bradycardia. Atropine reversed the effects of Dex on the expression of gap junction proteins.
*Conclusion:* Dex possibly altered the expression of gap junction proteins to slow down cardiac conduction velocity in the sinoatrial node.


* Corresponding author.
*E-mail:* 490173559@qq.com (Y. Zhong).

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## Introduction

Dexmedetomidine (Dex) is widely used for sedation in intensive care units and anesthesia settings. Dex can decrease the level of catecholamine transmitters and increase acetylcholine in the heart. It has a significant inhibitory effect on the functions of the Sinoatrial (SA) and Atrioventricular (AV) nodes,[1] which can reduce heart rate, cause bradycardia and has the potential to induce cardiac arrest.[2,3] Atropine can reverse those cardiac effects.

The main function of cardiac connexins is the metabolic and electrical coupling among myocytes, which is the structural basis for maintaining electrical impulses and closely related to the electrophysiological properties of the unique cardiac conduction system.[4,5] Connexin 30.2 (Cx30.2) and connexin 40 (Cx40) are mainly expressed in the SA and AV nodes, which are essential to maintain normal function of the cardiac conduction system.[6,7] Cx40 has high electrical conductivity, which facilitates the rapid conduction of electrical impulses through cardiomyocytes, to increase the heart rate. However, Cx30.2 has the opposite effect.

We hypothesized that dexmedetomidine-induced bradycardia is caused by the change in Cx30.2 and Cx40 in the SA node of rats. This study determined the expression of Cx30.2 and Cx40 in the SA node of rats with Dex-induced sinus bradycardia, investigated if atropine can reverse the changes in expression, and elucidated the possible associated mechanism of action.

## Methods

### Animals and protocols

All protocols were approved by the Animal Care Welfare Committee of Guizhou Medical University (n° 1800454). Our study was performed in the laboratory of Guizhou Medical University, and the animals were provided by Guizhou Medical University. Rats were fed for one week in the laboratory to adapt to the new environment, light and dark alternated for 12 hours a day. Healthy adult Sprague-Dawley rats (n = 80) weighing 240—300 g were randomly divided into five groups according to a random number table. Rats with abnormal electrical-cardiac function were excluded. According to other studies,[8,9] combined with our pre-experiment, we chose different doses of Dex in our experiment. The rats in group C (control group) were administered normal saline at a loading dose of 60 $mL.kg^{-1}.h^{-1}$ for 10 min and then at 10 $mL.kg^{-1}.h^{-1}$ for 120 min. In group $D_1$, the rats were administered Dex at a loading dose of 30 $\mu g.kg^{-1}$ for 10 min and then at 15 $\mu g.kg^{-1}.h^{-1}$ for 120 min. The rats in group $D_1A$ were administered Dex the same way as in group $D_1$; however, immediately after the administration of the loading dose of Dex, 0.5 mg atropine (Suicheng Pharmaceutical Co., Ltd., Henan, China) was administered intravenously and then at 0.5 $mg.kg^{-1}.h^{-1}$ for 120 min. In group $D_2$, the rats were administered Dex at a loading dose of 100 $\mu g.kg^{-1}$ for 10 min and then at 50 $\mu g.kg^{-1}.h^{-1}$ for 120 min. The rats in group $D_2A$ were administered Dex in the same way as group $D_2$, but immediately after the administration of the loading dose of Dex, 0.5 mg atropine was administered intravenously and then at 0.5 $mg.kg^{-1}.h^{-1}$ for 120 min.

### Experiments

Rats were anesthetized with sodium pentobarbital (50 $mg.kg^{-1}$, intraperitoneal injection). After the loss of righting reflexes, the animals were placed on their backs on a wooden board with their legs restrained by adhesive tape. Local anesthesia (0.5% lidocaine) was administered before femoral artery and vein catheterization. Small needle electrodes were inserted into the skin of the limbs and connected to the BL-420F information data acquisition and processing system (Chengdu Thaimeng software Co. Ltd., Chengdu, China) by crocodile clips to acquire standard lead II Electrocardiogram (ECG) data. The femoral artery was connected to the BL-420F by a polyvinyl chloride infusion extension tube to continuously monitor invasive arterial blood pressure. The femoral vein was connected to an infusion pump by an infusion extension tube to administer different drugs as per previously described procedures. The rats were administered continuous oxygen with oxygen flows of 2 $L.min^{-1}$ throughout the experiment, and $SpO_2$ was simultaneously monitored using a PM-9000 monitor (Mindraymedical international Co., Ltd., Shenzhen, China). During the experiment, the rats with Mean Arterial Pressure (MAP) < 70 mmHg, Heart Rate (HR) < 250 bpm after anesthesia, or ECG indicative of arrhythmia were excluded. The rats were divided into groups according to their original random numbers.

### Isolation of the SA node

Thoracotomy was performed on the rats after anesthetizing the chest area with 0.5% lidocaine, and the heart was rapidly excised and immersed in ice-cold Tyrode's solution in order to stop the heartbeat. The sinus node tissue was isolated as previously described[5] after further pruning of the well-exposed crista terminalis. The superior vena cava, inferior vena cava, crista auricularis dextra, and sulcus terminalis were used as the location markers of the SA node. The sulcus terminalis was considered the center and was removed from the back along the long axis of the venous sinus. The position located at the lower left point parallel to the sulcus terminalis at a distance of 5 mm was then removed by eye scissors. The upper edge of the sinus node was determined by the transection from above the crista auricularis dextra to the back of the crista auricularis dextra, and the lower edge was determined by the transection of the ostium venae cavae inferiors. Finally, the long strip of sinus node tissue was exposed. All biopsies had an approximate volume of 1 × 2 $mm^3$, and there were 16 samples in each group. Eight tissue samples were stored in individual Eppendorf safe-lock tubes prefilled with 0.5 mL RNA at 4°C overnight followed by storage at -80°C until quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) analysis. The other eight samples were preserved at -80°C for western blot assays.

### Time point monitoring

The BL-420F was used to monitor the HR and MAP of the rats throughout the experiment. HR and MAP were recorded at $T_0$ (just before Dex/saline infusion), $T_1$ (10 min after the beginning of Dex/saline infusion), $T_2$ (70 min after the

beginning of Dex/saline infusion), and $T_3$ (130 min after the beginning of Dex/saline infusion).

### RNA extraction and qRT-PCR

We employed magnetic bead separation to obtain mRNA from the SA node according to the manufacturer's instructions (Invitrogen, USA), which was then reverse transcribed into cDNA using a first strand Cdna synthesis kit (Roche, Switzerland). A fluorescent quantitative kit (Light cycler 480 sybr green 1 master, Roche, Switzerland) was used to complete the amplification reaction. qRT-PCR was carried out using the Real-Time PCR Detection System (CFX96, Bio-Rad Laboratories, Inc. USA). All the experiments were performed in triplicate, and mRNA levels were standardized to that of Glyceraldehyde-Phosphate Dehydrogenase (GAPDH). We examined the melting curves for each reaction to ensure the amplification of a single PCR product. Relative gene expression was determined by the comparative $C_T$ method. The primers used in the analysis are listed in Table 1.

### Western blotting

Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 8% tris-acetate gels before being transferred onto polyvinylidene difluoride membranes. After blocking with bovine serum albumin (5%), the membranes were incubated overnight at 4°C with rabbit polyclonal antibodies against mouse proteins Cx30.2 (1:1000; Abcam Trading Co., Ltd., Shanghai, China), Cx40 (1:2000; GeneTex Technologies Inc., USA), and GAPDH (1:8000; Abcam Trading Co., Ltd., Shanghai, China) as loading control. This was followed by incubation with goat anti-rabbit IgG secondary antibody (1:12000; Abcam Trading Co., Ltd., Shanghai, China) for 1h at 22 ± 2°C and washing of the membranes with tris-buffered saline containing tween 20. Reactive protein bands were detected using enhanced chemiluminescence and visualized using the Bio-Rad system. Bands from the resulting pictures were then quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

### Statistical analysis

Data were analyzed using SPSS 20.0 software and expressed as mean ± standard deviation. Statistical differences were assessed using one-way analysis of variance (ANOVA) and repeated measures analysis of variance. A $p$-value $< 0.05$ indicated statistical significance.

## Results

### Effects of Dex and Dex combined with atropine on HR in rats with sinus bradycardia

There were no significant differences in HR (beats/min) at $T_1$ (352 ± 40), $T_2$ (343 ± 42), and $T_3$ (335 ± 39) compared to HR at $T_0$ (350 ± 44) in group C ($p > 0.05$). HR was significantly lower at $T_1$ (244±25), $T_2$ (287 ± 29), and $T_3$ (262 ± 27) than at $T_0$ (355 ± 36) in groups $D_1$ ($p < 0.05$), whereas HR at $T_2$ was higher than at both $T_1$ and $T_3$ ($p < 0.05$). In group $D_2$, HR was lower at $T_1$ (212 ± 23), $T_2$ (215 ± 23), and $T_3$ (208 ± 23) than at $T_0$ (352 ± 38) ($p < 0.05$), and there was no difference in HR at $T_1$, $T_2$, and $T_3$ ($p > 0.05$). In group $D_1A$, HR was significantly lower at $T_1$ (241 ± 25) and at $T_3$ (315 ± 32) than at $T_0$ (350 ± 36) ($p < 0.05$), but no significant difference in HR was observed at $T_2$ (340 ± 35) ($p > 0.05$). HR was higher at $T_2$ and $T_3$ than at $T_1$ ($p < 0.05$). In groups $D_2A$, HR was significantly lower at $T_1$ (216 ± 22), $T_2$ (307 ± 31) and $T_3$ (286 ± 23) than at $T_0$ (361 ± 36) ($p < 0.05$), and HR was higher at $T_2$ and $T_3$ than at $T_1$ ($p > 0.05$). HR was significantly decreased at $T_{1\text{-}3}$ in groups $D_1$, $D_2$, and $D_2A$ compared to group C ($p < 0.05$). Only the HR at $T_1$ in group $D_1A$ was decreased compared to group C ($p < 0.05$) (Table 2).

### Changes in the expression level of Cx30.2 and Cx40 within the SA node of rats with sinus bradycardia

The qRT-PCR and western blot analyses revealed that the expression of Cx30.2 was significantly increased in groups $D_1$ (mRNA, 2.75 ± 0.51; protein, 1.43 ± 0.18), $D_2$ (mRNA, 4.84 ± 0.83; protein, 2.40 ± 0.06), and $D_2A$ (mRNA, 2.43 ± 0.23; protein, 1.43 ± 0.11) compared with group C (mRNA, 1.04 ± 0.03; protein, 1.00 ± 0.02) ($p < 0.05$), and was significantly higher in group $D_2$ than in group $D_1$ ($p < 0.05$). Moreover, mRNA and protein levels of Cx30.2 in groups $D_1A$ (mRNA, 1.29 ± 0.30; protein, 1.09 ± 0.04) and $D_2A$ were significantly decreased compared to those in the corresponding groups (groups $D_1$ and $D_2$, respectively) ($p < 0.05$), and was significantly higher in group $D_2A$ than in group $D_1A$ ($p < 0.05$). The qRT-PCR and western blotting results indicate that the mRNA and protein expression levels of Cx40 in groups $D_1$ (mRNA 0.63 ± 0.14; protein 0.73 ± 0.14) and $D_2$ (mRNA 0.34 ± 0.18; protein 0.56 ± 0.11) decreased compared to those in group C (mRNA, 1.11 ± 0.08; protein, 1.00 ± 0.04) ($p < 0.05$). The expression levels of Cx40 in Group $D_2$ were lower than those in group $D_1$ ($p < 0.05$). In addition, the expression levels of Cx40 in groups $D_1A$ (mRNA, 1.08 ± 0.12; protein, 0.96 ± 0.07) and $D_2A$ (mRNA, 0.96 ± 0.13, protein, 0.96 ± 0.13) were significantly increased compared to those in the

**Table 1** Primers for the targeted genes.

| Target gene | Primer sequence | Amplified length (bp) |
|---|---|---|
| GAPDH | Upstream primer- F: 5'TCTCTGCTCCTCCCTGTTC3'<br>Downstream primer- R: 5' ACACCGACCTTCACCATCT3' | 87 |
| Cx30.2 | Upstream primer- F: 5'AGCAGGAGGAGTTCGTGT3'<br>Downstream primer- R: 5'ACAGCCAGAAGCGGTAGT 3' | 96 |
| Cx40 | Upstream primer- F: 5′ACGTCTGCAGCATTGTCATC-3'<br>Downstream primer- R: 5′CCCAGGTGGTAGAGTTCAGC-3′ | 147 |

**Table 2** Changes in Heart Rate (HR) of rats with dexmedetomidine-induced sinus bradycardia.

| Monitoring parameter | Group | T0 | T1 | T2 | T3 |
|---|---|---|---|---|---|
| HR (beats/min) | C | 350 ± 44 | 352 ± 40 | 343 ± 42 | 335 ± 39 |
| | D1 | 355 ± 36 | 244 ± 25[a,b] | 287 ± 29[a,b,c] | 262 ± 27[a,c] |
| | D1A | 350 ± 36 | 241 ± 25[a,c] | 340 ± 35[b] | 315 ± 32[a,b] |
| | D2 | 352 ± 38 | 212 ± 23[a,c] | 215 ± 23[a,c] | 208 ± 23[a,c] |
| | D2A | 361 ± 36 | 216 ± 22[a,c] | 307 ± 31[a,b,c] | 286 ± 23[a,b,c] |

In group C (control group), rats were administered normal saline. In groups $D_1$ and $D_1A$ rats were administered dexmedetomidine at a loading dose of 30 $\mu g.kg^{-1}$ for 10 min and then at 15 $\mu g.kg^{-1}.h^{-1}$ for 120 min. In groups $D_2$ and $D_2A$, rats were administered dexmedetomidine at a loading dose of 100 $\mu g.kg^{-1}$ for 10 min and then at 50 $\mu g.kg^{-1}.h^{-1}$ for 120 min. Moreover, in groups D1A and D2A, rats were administered atropine at a loading dose of 0.5 mg and then at 0.5 $mg.kg^{-1}.h^{-1}$ for 120 min. Compared to $T_0$.

[a] $p < 0.05$; compared to $T_1$.

[b] $p < 0.05$; compared to group C.

[c] $p < 0.05$. (Data are presented as mean ± standard deviation, n = 16 per group).

corresponding groups (groups $D_1$ and $D_2$, respectively) ($p < 0.05$) (Figs. 1 and 2).

## Discussion

The heart is innervated by sympathetic and vagal nerves. Among the factors that influence the functions of SA and AV nodes, the autonomic nervous system plays the most important role.[10] A previous study reported that Dex, a highly selective $\alpha$2-receptor agonist, caused heart rate alterations via the suppression of the activities of the peripheral and central sympathetic nerves, thereby inducing vagal-dominant conditions.[11] With the stimulation of $\alpha$2-receptor binding in the locus coeruleus, Dex inhibits the release of neuronal norepinephrine and blocks the descending transmission of sympathetic nerve activity, thereby reducing adrenaline release from the adrenal medulla. In addition, Dex decreases sympathetic tone by inhibiting norepinephrine release from sympathetic terminals in the periphery, resulting in the reduction in blood pressure and HR.[12,13] In recent years, animal experiments have shown that Dex can directly activate the cardiac vagal nerve. Histocytological studies have also demonstrated the presence of $\alpha$2-adrenergic receptors in the ambiguus nucleus, dorsal nucleus, and tractus solitarius nucleus of the medulla oblongata.[8,9] Sharp et al.[14] reported that Dex selectively decreased both GABAergic and glycinergic inhibitory input to the nucleus ambiguus and cardiac vagal neurons, with no significant effect on excitatory input. Decreasing inhibitory neurotransmission to cardiac vagal neurons results in an increase in the excitability of parasympathetic neurons that project to the

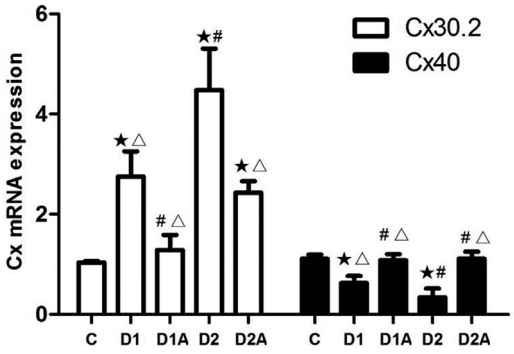
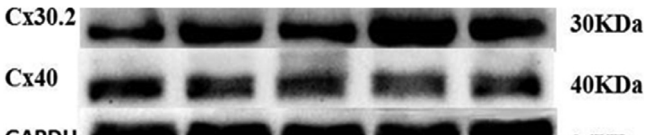



**Figure 1** mRNA expression of Cx30.2 and Cx40 in rats with dexmedetomidine-induced sinus bradycardia. C (control group), the rats were administered normal saline. In groups $D_1$ and $D_1A$, the rats were administered Dex at a loading dose of 30 $\mu g.kg^{-1}$ for 10 min and then at 15 $\mu g.kg^{-1}.h^{-1}$ for 120 min. Groups $D_2$ and $D_2A$ rats were administered Dex at a loading dose of 100 $\mu g.kg^{-1}$ for 10 min and then at 50 $\mu g.kg^{-1}.h^{-1}$ for 120 min. Moreover, the rats in groups $D_1A$ and $D_2A$ were administered atropine at a loading dose of 0.5 mg and then at 0.5 $mg.kg^{-1}.h^{-1}$ for 120 min. ★$p < 0.05$ vs. Group C, #$p < 0.05$ vs. Group $D_1$; △$p < 0.05$ vs. Group $D_2$. Data are presented as mean ± standard deviation, n = 8 per group).



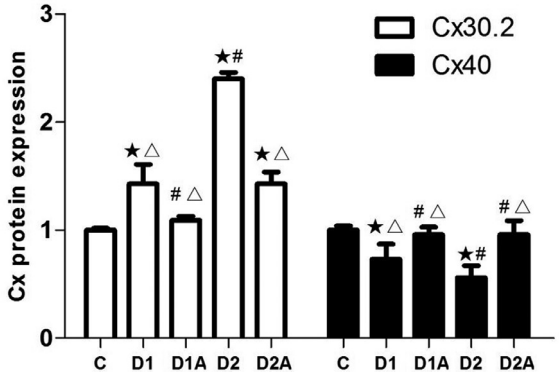




**Figure 2** Protein expression of Cx30.2 and Cx40 in rats with dexmedetomidine-induced sinus bradycardia. In Group C, the rats were administered normal saline. The rats in groups $D_1$ and $D_1A$ were administered Dex at a loading dose of 30 $\mu g.kg^{-1}$ for 10 min and then at 15 $\mu g.kg^{-1}.h^{-1}$ for 120 min. In groups $D_2$ and $D_2A$, the rats were administered Dex at a loading dose of 100 $\mu g.kg^{-1}$ for 10 min and then at 50 $\mu g.kg^{-1}.h^{-1}$ for 120 min. Moreover, in groups $D_1A$ and $D_2A$, the rats were administered atropine at a loading dose of 0.5 mg and then at 0.5 $mg.kg^{-1}.h^{-1}$ for 120 min. ★$p < 0.05$ vs. Group C, #$p < 0.05$ vs. Group $D_1$; △$p < 0.05$ vs. Group $D_2$. Data are presented as mean ± standard deviation, n = 8 per group.

heart. Shimizu et al.[8,15] demonstrated that 100 $\mu g.kg^{-1}$ medetomidine (a racemic mixture of two stereoisomers, Dex and levomedetomidine, with Dex as the active enantiomer) activates the cardiac vagal nerve via the modulation of baroreflex control of the central nervous system.

A bradycardia rat model is considered to be successfully established if the HR decreases by > 30% and is stable for 30 min.[16] Shimizu et al.[8,9] demonstrated that both 10 and 100 $\mu g.kg^{-1}$ medetomidine significantly decreased norepinephrine levels in cardiac dialysis. Intravenous medetomidine (100 $\mu g.kg^{-1}$) significantly increased the cardiac dialysate acetylcholine concentrations both in rabbits and rats and had an effect equivalent to electrical vagal stimulation at 10 Hz (20 Hz electrical vagal stimulation may cause AV block or sinus arrest). We chose a loading dose of Dex (30 $\mu g.kg^{-1}$) as the low-dose group, which can just lead to HR decrease > 30% in rats. It is the threshold dose to make the model of sinus bradycardia successfully constructed. We chose a loading dose of Dex (100 $\mu g.kg^{-1}$) as the high-dose group. It is the threshold dose to make the HR decreased to a liminal value in rats, the heart rate did not decrease even as the drug dose increased.

A large number of studies have revealed that different types of gap junction channels have different conductance and that their ionic selectivity and permeation properties remain different.[7] In the cardiac conduction system, Cx40 and Cx43 have high unitary conductance, whereas Cx30.2 and Cx45 have low unitary conductance. It is now known that the SA and AV nodes are specialized tissues with slow impulse propagation that only express Cx30.2 (9 pS) and Cx45 (32 pS).[17] The co-expression of Cx30.2 and Cx40 within the AV node has been detected in mice. In addition, the homotypic gap junction channels formed by Cx40 have high unitary conductance (180 pS), whereas the Cx43/Cx45 heterotypic gap junctions formed by Cx30.2/Cx40 have relatively low unitary conductance (18 pS).[18] Significant changes in ECG and the indicators of the conduction function of the AV node have been reported in Cx30.2 and Cx40 gene-deficient mice.[17,19] Cx30.2, as an important marker of slow conduction, and Cx40, as an important marker of rapid conduction, contribute to the modulation of electrical impulse propagation in different areas of the AV node,[4,20] thereby maintaining normal AV conduction.

Compared to baseline HR, there was a reduction in the HR of rats in groups $D_1$ and $D_2$ by 31% and 40%, respectively, but neither AV block nor sinus arrest was observed. The MAP of the two experimental groups was significantly lower than that of the control group, and the expression levels of Cx30.2 in groups $D_1$ and $D_2$ were significantly higher than in group C. In addition, the expression levels of Cx40 in groups $D_1$ and $D_2$ were significantly lower than that in group C. Gap junctions are membrane channels that mediate the cell-to-cell movement of ions and small metabolites. In the heart, gap junctions play an important role in impulse conduction, and intercellular coupling disorders are an important cause of arrhythmia. The mechanism by which Dex influences the expression of Cx30.2 and the involvement of increased vagal activity have remained unknown.

The induction of vagal-dominant conditions by suppressing the activity of sympathetic nerve and directly increasing the activity of cardiac vagal nerve are widely recognized as the mechanisms by which Dex induces change in autonomic balance. We used atropine, a competitive and reversible antagonist of muscarinic acetylcholine receptors, to block $M_2$ receptors in the SA node. According to Jammes,[21] 0.5 mg atropine can completely block cholinergic neurotransmission in the heart of rats, thereby essentially relieving the inhibition of the vagus nerve on the heart. The results indicate that the expression of Cx30.2 was significantly reduced in groups $D_1A$ and $D_2A$ compared to groups $D_1$ and $D_2$, and the expression of Cx40 was significantly increased in groups $D_1A$ and $D_2A$ compared to groups $D_1$ and $D_2$. It can be inferred that the increased vagal activity may be one of the factors responsible for the changes in the levels of expression of Cx30.2 and Cx40 within the SA node in rats with Dex-induced sinus bradycardia. However, the HR in group $D_2A$ was still significantly lower than that in group C, and the expression of Cx30.2 in group $D_2A$ was significantly higher than that in group C. Based on our experimental results, we hypothesize that other mechanisms may be associated with the effect of high-dose Dex on the expression of Cx30.2,[22] and a further study is needed to identify the factors that are associated with the changes in the expression of Cx30.2.

Our study showed that bradycardia caused by prolonged (about 2h) Dex treatment may lead to changes in the expression of connexins in the SA node. In recent years, some studies have reported that Dex may cause cardiac arrest.[2,3] There is a study showing that changes of connexin expression in the heart can lead to abnormal conduction of electrical impulses, leading to arrhythmias and even sudden death.[23] The sinoatrial node is the main pacemaker of the heart, and its dysfunction may be more likely to cause arrhythmias. Considering the present findings, we suggest caution in the administration of Dex in patients with an impaired cardiac conduction system, especially those displaying SA node dysfunction. If Dex-related bradycardia occurs, atropine could be used to reduce the incidence of cardiac adverse events.

There were some limitations in our study. First of all, the cause of protein change in rats was not clear. Change of neurotransmitters in the sinoatrial node, or the direct effects of Dex on the sinoatrial node were all possible reasons. Secondly, in clinical anesthesia, although administration of Dex was in low dosage, the incidence of bradycardia was high. The purpose of our study was to observe the change in gap junction protein connexins in the sinoatrial node of bradycardia in rats. In order to ensure rats presented bradycardia, we choose relatively large doses of Dex. It is possible that the effects of Dex in humans could be somewhat different regarding the expression of the same proteins.

## Conclusions

In summary, the expression of the low-conductive gap junction protein, Cx30.2, within the SA node was increased in rats with Dex-induced sinus bradycardia, and the expression of the high-conductive gap junction protein, Cx40, was decreased. The change in the expression of gap junction proteins, Cx30.2 and Cx40, is one of the causes of cardiac sinus bradycardia, and autonomic nervous activity is involved in the regulation of these proteins.

## Conflicts of interest

The authors declare no conflicts of interest.

## Acknowledgments

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