Impacts and mechanisms of dexmedetomidine HCl on heart rate in rabbit with bilateral vagotomy or sympathectomy.

Jie Wu¹, Enjun Lei¹*, Jiegang Zhou², Doudou Zhao²

¹Department of Anesthesiology, the First Affiliated Hospital of Nanchang University, Nanchang 330006, PR China
²Department of Medicine, Graduate School, Nanchang University, Nanchang 330006, PR China

Abstract

The aim of the current study was to investigate the impacts of dexmedetomidine HCl (Dex) on the heart rate in the rabbits with bilateral vagotomy or sympathectomy. 30 healthy male New Zealand white rabbits, weighing 2~2.5 kg, were randomly divided into three groups (n=10): the experimental group (group V) was abscessed the vagus nerves, the de-sympathetic nerve group (group S), and the control group (group C, with autonomic nerves preserved). After being anesthetized, group V and S were performed bilateral vagotomy and sympathectomy, respectively, but group C were retained the bilateral autonomic nerves. The three groups were all intravenously infused Dex 3 µg/kg within 10 min. The heart rates (HR) and the arterial acetylcholine (Ach) contents in the three groups were recorded at different time points (after anesthesia (T0), after neurectomy (T1), and 10 min after Dex infusion (T2). Group V and S significantly decreased HR while significantly increased Ach at T2 (p<0.05). Intravenous injection of Dex can reduce HR in rabbits; after cutting off the bilateral vagus nerves or sympathetic nerves, the release of Ach is increased, so HR in rabbits is further declined.

Keywords: Dexmedetomidine, Heart rate, Autonomic nervous system, Acetylcholine.

Accepted on August 10, 2017

Introduction

Dexmedetomidine HCl (Dex) is a highly selective α2-adrenergic receptor agonist widely used in perioperative sedation and analgesia due to its rapid onset, short action duration, and can strongly inhibit body's stress responses. Its main clinical side effects are bradycardia and hypotension [1]. With the improvements of medical environment and healer surgical techniques day by day, as well as deeper understanding about human physiologic and pathologic knowledge, many elderly patients, critically ill patients, and those with surgical contraindications previously have been included within the scope of the operation [2,3]. Patients with high vagal tone, diabetes, hypertension, elderly, or liver or renal dysfunction may easily occur bradycardia, or even sinus arrest when using Dex [4]. Despite extensive literatures have reported the applications of Dex, about more than 90% of these studies is focused on its clinical efficacies and organ protective effects [5,6]. However, studies about Dex-related bradycardia and atrioventricular block are rarely reported, and it has not attracted enough attention from most anesthesiologists yet.

Currently, clinical causes for bradycardia are mainly vagus nerve hyperactivity and sinus pacing and conduction abnormalities. Bradycardia caused by Dex is closely related to the state of autonomic nervous system [7-10]; this study incised the bilateral vagus nerves or bilateral sympathetic nerves of New Zealand white rabbits, and then observed the impacts of Dex on the heart rate and Ach, aiming to investigate its possible mechanisms of slowing the heart rate.

Materials and Methods

Animal model and grouping

A total of 30 healthy male New Zealand white rabbits were provided by Wuhan Wanqianjiaxing Biotechnology Co. Ltd. (License number: No.42010000001197), weighing 2~2.5 kg. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Nanchang University. All the rabbits were fed one week for the adaption in strict accordance with the “animal feeding, management and usage guidelines”; the laboratory temperature was controlled within 22~24°C with the relative humidity as 50% to 60%. The environment was kept highly clean, and the rabbits can freely access to water and food. Then, the computer-generated random number table was used to randomly divide these rabbits into three groups (n=10), namely group V, group S, and group C. 1% pentobarbital sodium was injected along the rabbit ear vein (3 ml/kg, 5 g, batch number: 8310052, Beijing Solarbio, place of production: Germany), and after being anesthetized, each rabbit was fixed onto one dog-
rabbit laboratory platform. One DATEX-OHMEDA multifunctional monitor was used to monitor electrocardiogram (ECG) and heart rate; the ear vein was then catheterized and fixed. After cut off the neck fur and disinfected, the trachea of each rabbit was longitudinally incised and exposed so as to expose the common carotid artery; the left common carotid artery was then catheterized for monitoring the arterial blood pressure. As for the rabbits in group V, the coarse and fine gray neural stems seen beside the common carotid artery are the vagus nerve and aortic nerve, respectively. After the bilateral vagus nerves were cut off, 3 μg/kg Dex was intravenously infused (2 ml/200 μg, batch number: 15040432, Jiangsu Hengrui Medicine Co., Ltd., Jiangsu, China) within 10 min. Group S was isolated and exposed along the common carotid artery until the bifurcation, namely the bifurcation of internal and external carotid artery; the carotid sheath was then stripped, and the following careful blunt dissection deviated toward the internal carotid artery can reveal the superior cervical (anterior cervical) sympathetic node attaching to the artery, one gray-red oval swollen body, namely the sympathetic nerve. After cutting off the bilateral sympathetic nerves, 3 μg/kg Dex was intravenously infused within 10 min. Group C were separated the bilateral autonomic nerves but not cut off, followed by the intravenous infusion of 3 μg/kg Dex within 10 min. Each group was oxygenized, and the death case was removed from the group.

Specimen collection and processing

A 4 ml of blood was sampled from the common carotid artery of each group immediately after anesthesia (T0), and then divided into two parts (2 ml in each part); after stood at room temperature (22~24°C) for 1 h, the blood was centrifuged for 20 min (4°C, 13000 rpm), and the plasma was then stored at -20°C. Arterial blood was sampled from group V immediately after cutting off the bilateral vagus nerves (T1) and 10 min after the intravenous infusion of Dex (T2). Arterial blood was sampled from group S immediately after cutting off the bilateral sympathetic nerves (T1) and 10 min after the intravenous infusion of Dex (T2). Arterial blood was sampled from group C immediately after separating the bilateral nerves (T1) and 10 min after the intravenous infusion of Dex (T2). The processing procedures of blood samples of each group at T0, T1, and T2 were the same.

Observation indexes

The change rates of heart rates (HR) of each group at T0, T1, and T2 were observed and recorded. The serum Ach was measured using the colorimetric detection method (Ach Assay Kit, Nanjing Jiancheng Bioengineering Institute, place of production: China).

Statistical analysis

SPSS22.0 software was used for the statistical analysis, and all the measurement data were express as x ± s; the intergroup comparison used the t test, and the intragroup comparison used the analysis of variance for the repeated measurement data, with p<0.05 considered statistically significant.

Results

General data

The rabbits in the three groups were generally in good conditions before the experiment, and there was no statistically significant difference in their weights (p=0.13). The amount of sodium pentobarbital had not statistically significant difference (p=0.47). During the experiment, no special events or abnormal ECG occurred.

The comparison of HR

HR in group V at T2 (194.10 ± 21.44) showed statistically significant difference with that in group C (219.20 ± 15.56) (p<0.05), but HR in group V at T0 and T1 showed no statistically significant difference with those in group C (p>0.05). The intragroup comparison of HR in group V at T2 revealed that HR was significantly decreased, and the difference was statistically significant (p<0.05), but that at T1 was increased while no statistically significant difference was discovered (p>0.05, Table 1).

Table 1. Comparison of each index between group V group C at different time points (n=10, x ± s).

<table>
<thead>
<tr>
<th>Group</th>
<th>HR f/(beats·min⁻¹)</th>
<th>Ach ρ/(μg·ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T0</td>
<td>222.60 ± 13.40</td>
<td>10.51 ± 2.67</td>
</tr>
<tr>
<td>T1</td>
<td>234.90 ± 23.39</td>
<td>9.23 ± 2.00</td>
</tr>
<tr>
<td>T2</td>
<td>194.10 ± 21.44</td>
<td>13.34 ± 3.69‡</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T0</td>
<td>214.10 ± 21.44</td>
<td>9.42 ± 0.83</td>
</tr>
<tr>
<td>T1</td>
<td>225.60 ± 14.98</td>
<td>9.20 ± 0.88</td>
</tr>
<tr>
<td>T2</td>
<td>219.20 ± 15.56</td>
<td>10.02 ± 2.18</td>
</tr>
</tbody>
</table>

Note: ‡p<0.05, intragroup comparison with the baseline value at T0; †p<0.05, compared with the value in group C at the same time point.

HR in group S at T1 (217.10 ± 20.55) and T2 (188.70 ± 23.66) showed statistically significant difference with those in group C (245.60 ± 14.97) and (219.20 ± 15.56), respectively (p<0.05). The intragroup comparison of group S between T1 (217.10 ± 20.55) and T0 (234.90 ± 23.39) showed statistically significant difference (p<0.05); the intragroup comparison of group S between T2 (188.70 ± 23.66) and T0 (234.90 ± 23.39) showed statistically significant difference (p<0.05, Table 2).

The comparison of Ach

Ach in group V at T2 (13.34 ± 3.69) showed statistically significant difference with that in group C (10.02 ± 2.18) (p<0.05), but the intergroup comparison at T0 and T1 showed
Impacts and mechanisms of dexmedetomidine HCl on heart rate in rabbit with bilateral vagotomy or sympathectomy

no statistically significant difference. Ach in group V showed statistically significant difference between T2 (13.34 ± 3.69) and T0 (10.51 ± 2.67) (p<0.05); although Ach at T1 was slightly reduced, no statistically significant difference was discovered (p>0.05, Table 1).

Ach in group S at T2 (12.55 ± 2.54) showed statistically significant difference with that in group C (10.02 ± 2.18) (p<0.05). The intragroup comparison of group S between T2 (12.55 ± 2.54) and T0 (9.94 ± 3.05) showed statistically significant difference (p<0.05), but the content at T1 was slightly increased, and showed no statistically significant difference (Table 2).

Table 2. Comparison of each index between group S group C at different time points (n=10, x ± s).

<table>
<thead>
<tr>
<th>Group</th>
<th>HR f/(beats·min⁻¹)</th>
<th>ACH ρ/(μg·ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T₀</td>
<td>234.90 ± 23.39</td>
<td>9.94 ± 3.05</td>
</tr>
<tr>
<td>T₁</td>
<td>217.10 ± 20.55</td>
<td>10.69 ± 4.33</td>
</tr>
<tr>
<td>T₂</td>
<td>188.70 ± 23.66</td>
<td>12.55 ± 2.54</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T₀</td>
<td>214.10 ± 21.44</td>
<td>9.42 ± 0.83</td>
</tr>
<tr>
<td>T₁</td>
<td>225.60 ± 14.98</td>
<td>9.20 ± 0.88</td>
</tr>
<tr>
<td>T₂</td>
<td>219.20 ± 15.56</td>
<td>10.02 ± 2.18</td>
</tr>
</tbody>
</table>

Note: 'p<0.05, intragroup comparison with the baseline value at T₀; 'p<0.05, compared with the value in group C at the same time point.

Discussion

Dex is a highly-pure α2 receptor agonist, and clinical observations suggest that the intravenous injection of Dex may produce very ideal natural sleep-like effects [11], but it can also cause immediate heart rate decreasing, bradycardia, or even cardiac arrest [12,13]. Intravenous anesthetic drug-induced HR changes are usually caused by their interference with the autonomic nervous system. The autonomic nervous system can be divided into the sympathetic system and the vagal system, which impact heart rate through releasing certain chemical mediators. The sympathetic nerve can release norepinephrine and epinephrine so as to act on the receptors, and the vagus nerve can release Ach from its end, thus acting on the cardiac M receptor and impacting the electrical activities of the heart. Ach can delay the sinus diastolic auto-depolarization, increase the repolarization current, prolong the time interval of the action potential before re-reaching the threshold, thus slowing down HD. Ach may also extend the refractory period of atrioventricular node and Purkinje fibers so as to slowing its conduction [14].

Many scholars have investigated the mechanisms of Dex targeting its roles of slowing HR, but no conclusion has ever been made. Some studies [15,16] think that α2 receptor agonists can act on the presynaptic membrane of peripheral nerve endings, thus inhibiting the release of norepinephrine, and this may be its primary mechanisms of slowing down HR. Some other literatures [17-19] also think that Dex can inhibit the sympathetic activities, and enhance the activities of the vagus nerve, thus slowing down HR.

This study observed that when the rabbits in group V were cut off the bilateral vagus nerves, their HR increased significantly, and was higher than that in group C at the same time point, indicating that when the vagus innervation was lost, the sympathetic nerve exhibited its predominance. However, after being continuously infused Dex, the rabbits in group V appeared immediate HR decreasing, and HR at T2 (194.10 ± 21.44) was significantly lower than that in group C (219.20 ± 15.56). The reason that cause of heart rate decrease due to involve vascular smooth muscle α2B receptor during the initial injection phase, resulting in a brief increase in blood pressure and a decrease in reflex heart rate. Followed by continuous infusion, anti-sympathetic effect in the center, and reduced the concentrations of norepinephrine and adrenaline in plasma, resulting in a decrease of heart rate. The Ach content in group V was slightly decreased than group C after vagotomy, but the continuous infusion of Dex significantly increased the Ach content (13.34 ± 3.69) than group C (10.02 ± 2.18). The mechanisms may be after the bilateral vagus nerves were cut off, Dex promoted the release of Ach by peripheral nerve pathways [20,21]. Even though there was litter evidence that Dex can increase the activity of the vagus nerve, studies suggest that Dex can reduce the neurotransmitters, which are spread from the cerebral ambiguous nucleus and can inhibit the vagus nerve, but Dex do not increase the excitatory neurotransmitters [1,22], consistent with our results. Unfortunately, this article can only prove that the concentration of Ach can still increase after neuroectomy, but cannot prove that vagus nerve can be directly excited by Dex.

After the bilateral sympathetic nerves in group S were cut off, HR immediately slowed, but the Ach content showed no significant change. Continuously infusing Dex continuously declined HR, and that at T2 (188.70 ± 23.66) was significantly lower than group C (219.20 ± 15.56), but the Ach content (12.55 ± 2.54) was significantly higher than group C (10.02 ± 2.18). Studies have shown that Dex can inhibit the activities of sympathetic nerve, thus slowing HR [23,24]; this study observed that even the bilateral sympathetic nerves were cut off, the intravenous infusion of Dex still caused further slowing of HR, inconsistent with the above literatures. The further decreased HR observed in this study may be related to the significantly increased Ach content in vivo.

The results of this study showed that after the bilateral vagus nerves or the bilateral sympathetic nerves were cut off, intravenously injecting Dex can decrease HR but increase the Ach content, and the amplitudes of HR decreasing and Ach content increasing are higher than those in group C, indicating that Dex can reduce HR in the rabbits with bilateral vagotomy or sympathectomy. Furthermore, regardless of vagotomy or sympathectomy, Dex can increase the serum Ach content in the rabbits, so it can be inferred that Dex can also increase the
release of Ach via non-autonomous nerve pathways and it will be the direction of our further experiment.

Conclusions
Intravenously injecting Dex can decrease HR in New Zealand rabbits, and after vagotomy or sympathectomy, Ach can be significantly upregulated, thus leading to a further decline of HR in these New Zealand rabbits. The mechanisms may be explained as after cutting off the vagus nerve or sympathetic nerve, Dex can significantly increase the release of Ach via non-autonomous nerve pathways.

References
17. Philipp KE, Bateman RI, Mendelowitz D. Clonidine, an alpha2-receptor agonist, diminishes GABAergic neurotransmission to cardiac vagal neurons in the nucleus ambiguus. Brain Res 2010; 1347: 65-70.


*Correspondence to
Enjun Lei
Department of Anesthesiology
The First Affiliated Hospital of Nanchang University
PR China