VAGAL MODULATION OF HEART RATE IN THE nNOS KNOCKOUT MOUSE IN VITRO

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INTRODUCTION

The role of nitric oxide (NO) synthesized from neuronal NO-synthase (nNOS) in the vagal modulation of heart rate (HR) is controversial. Pharmacological inhibitors of nNOS have been reported to significantly attenuate1 or to have little effect on the decrease in heart rate with vagal nerve stimulation (VNS).2,3 This effect may depend on the expression of NOS and bioavailability of NO.

AIMS of the study
(1) To identify nNOS within the SA nodal innervation of the mouse heart.
(2) To compare the heart rate responses to vagal stimulation in isolated right vagus/double atria preparations from wild-type homozygous (WT; nNOS+/-), heterozygous (nNOS+/-), and nNOS knockout (nNOS-/-) mice.
(3) To assess the role of upstream and downstream NO-cGMP pathways in cholinergic modulation of HR.

METHODS

Animals
All animals were 3-4 month-old males. WT, nNOS+/- and nNOS-/- genotypes were all confirmed in tissue samples taken from tail clippings.

Anatomy
The right atrium and slices of the hypothalamus (4µm) were processed for immunohistochemistry. Immunoreactivity was revealed by the chromogen substrate diaminobenzidine with hydrogen peroxide.

Physiology and Pharmacology
A double atrial/right vagal preparation was dissected free, placed into an organ bath containing mouse physiological saline bubbled with carbogen (95% O2, 5% CO2) and connected to an isometric force transducer. Heart rate was triggered from contraction. The change in heart rate with vagal stimulation for 3 s or bath-applied CCh (10^-8 - 10^-4 M) was measured. Drugs were added to the organ bath after control protocols were completed.

RESULTS

Anatomy - nNOS in right atrium & hypothalamus

PHYSIOLOGY - IN VITRO VAGAL HR RESPONSES

* There were no differences in ventricular body weight ratios.
* Baseline HR was elevated in nNOS-/- compared to WT atria (Table 1).
* There was no difference in the magnitude of the HR response to vagal stimulation (Figure 2).
* The rate of decline in HR (TT50%) with vagal stimulation was significantly slower in nNOS-/- than WT mice (Figure 2 & Table 1).

Table 1 Baseline HR, TT50% (3 & 5Hz) in WT, nNOS-/- and nNOS+/-.

<table>
<thead>
<tr>
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<th>Baseline HR (bpm)</th>
<th>3Hz TT50% (s)</th>
<th>5Hz TT50% (s)</th>
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<tbody>
<tr>
<td>WT (n=6)</td>
<td>322 ± 7*</td>
<td>7.03 ± 0.38*</td>
<td>5.74 ± 0.23*</td>
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<tr>
<td>nNOS -/- (n=6)</td>
<td>360 ± 7*</td>
<td>7.03 ± 0.38*</td>
<td>5.74 ± 0.23*</td>
</tr>
<tr>
<td>nNOS +/- (n=15)</td>
<td>338 ± 8</td>
<td>6.66 ± 0.18</td>
<td>5.56 ± 0.33</td>
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* There were no statistical differences in the IC50 concentrations of bath-applied carbamylcholine (CCh) for heart rate responses in WT and nNOS-/- (Table 2).

Table 2 IC50 values for CCH HR Response (10^-8-10^-4 M) in WT vs. nNOS-/-.

<table>
<thead>
<tr>
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<th>IC50 for CCH HR Response (10^-8 M)</th>
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<tbody>
<tr>
<td>WT (n=12)</td>
<td>nNOS +/- (n=16)</td>
</tr>
<tr>
<td>nNOS -/-</td>
<td>6.87 ± 0.39</td>
</tr>
<tr>
<td>nNOS -/-</td>
<td>6.00 ± 0.37</td>
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* There were no differences in the IC50 concentrations of CCh for heart rate responses in WT and nNOS-/- (Table 2).

CONCLUSION

The findings of this study suggest that nNOS present in the sympathetic nerves supplying the atria produces NO that facilitates presynaptic cholinergic neurotransmission, and contributes to the decrease in heart rate in response to vagal nerve stimulation.

References