Effects of Hexamethonium on Bradycardiac Responses to Brain Ischemia in the Rabbit

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Abstract The present study investigated the bradycardiac responses to brain ischemia for approximately 30 s before and after intravenous administration of hexamethonium (C6, 15 mg/kg) in urethane-anesthetized spontaneously breathing rabbits. The brain ischemia was performed by clamping both common carotid arteries in rabbits whose vertebral arteries were previously occluded. The brain ischemia caused bradycardia, pressor response and apnea. Administration of C6 blocked the bradycardia evoked by brain ischemia and reduced pressor response at the initial period after the onset of brain ischemia. The brain ischemia-induced apnea was not significantly altered by C6-treatment. In a separate series of experiments, we examined the effects of C6 on the response of heart rate (HR) to vagal stimulation in rabbits following unilateral vagotomy. Electrical stimulation of the peripheral end of the cut left vagus nerve that selectively activated myelinated fibers caused the bradycardia and this effect was entirely blocked by administration of C6. When the intensity of stimulus to activate both myelinated and non-myelinated fibers was increased, part of the bradycardia was retained following C6 administration. These results suggest that the brain ischemia-induced bradycardia is totally mediated through the activation of myelinated efferent fibers in the vagus nerve.

Key words: brain ischemia, hexamethonium, myelinated vagal efferent fibers, rabbit, vagal efferent stimulation.

Interruption of the blood supply to the head elicits a characteristic cardio-respiratory effect, for example, pressor response [1–4], bradycardia [3–5], and apnea [4, 5]. In anesthetized spontaneously breathing rabbits, section of both aortic nerves greatly diminishes or completely abolishes the bradycardia evoked by global brain ischemia which is produced by occluding both common carotid and vertebral arteries [6–8] and vagotomy invariably abolishes the brain ischemia-

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induced bradycardia [4, 9]. As regards the magnitude of brain ischemia-induced reflex bradycardia, recent evidence has shown that the ability of aortic C fibers is much greater than that of aortic A fibers [10]. However, it is still unknown whether the brain ischemia-induced reflex bradycardia is mediated by the activation of either myelinated efferent fibers only or both myelinated and non-myelinated efferent fibers in the vagus nerve.

Electrical stimulation of the peripheral cut-end of the vagus nerve is known to cause a decrease in heart rate (HR). In the rabbit the stimulation of non-myelinated fibers in the vagus nerve increases the magnitude and duration of the bradycardia produced by the myelinated fiber activation [11, 12]. In addition, the effect of myelinated fiber activation on HR is blocked by the treatment with hexamethonium (C₆) which does not significantly affect the bradycardia induced by recruitment of non-myelinated fiber activation [11, 12]. The results lead to the suggestion that the actions of myelinated and non-myelinated cardiac vagal efferent fibers on bradycardia are differentiated by administration of C₆ in the rabbit.

To elucidate the roles of myelinated and non-myelinated vagal efferent fibers in the responses of bradycardia to brain ischemia, we re-examined the effects of electrical stimulation of the left or right vagus nerve to activate either myelinated fibers or both myelinated and non-myelinated fibers on the HR responses before and after administration of a nicotinic blocker, C₆, or a muscarinic receptor blocker, atropine. We then examined the HR responses to brain ischemia for approximately 30 s before and after administration of C₆.

METHODS

Twenty-one rabbits weighing 2.5 - 3.5 kg were anesthetized with urethane (1 g/kg, i.p.). Additional doses of this anesthetic agent (0.2 - 0.3 g/kg, i.p.) were administered as required. The trachea was cannulated low in the neck to obtain spontaneous breathing with room air, and the larynx and esophagus were reflected to expose both carotid artery bifurcations. After administration of heparin (500 U/kg) into the ear vein, the femoral artery was cannulated for measurement of arterial pressure (AP) with a pressure transducer and for recording of heart rate (HR) with a cardiotachometer. The femoral vein was also cannulated for infusion of drugs and of dextran or Locke's solution. The tracheal side-pressure (P_{trach}) used as an index of respiratory movement was measured by connecting the tracheal cannula to a differential transducer. The rectal temperature was maintained at around 37°C by a heating pad.

The left or right vagus nerve was exposed and sectioned close to the nodose ganglion. A paraffin pool was made with skin flaps. The desheathed peripheral cut-end of one vagus nerve at the C₂ level was placed on bipolar stimulating electrodes. Then, bipolar recording electrodes were caudally applied to the cervical vagus nerve. The distance between these electrodes was approximately 30 mm. Conventional stimulation of the vagus nerve was composed of square-wave pulses.
to evoke a maximal volley in myelinated fibers or volleys in both myelinated and non-myelinated fibers. The evoked action potentials of the vagus nerve were displayed on an oscilloscope screen and photographically recorded. The evoked volleys were monitored throughout the experiments.

Schematic illustration of the technique of the global brain ischemia is shown in Fig. 1. To interrupt the arterial blood flow at the third and fourth cervical vertebrae, holes were made on the ventral surface of the transversal processes by using a dental drill. This procedure was carefully performed to prevent bleeding from the vertebral artery and vein. Then, a piece of a gauze stick stiffened by bone wax was forcefully inserted into each hole. After irreversible interruption of the vertebral artery, the global brain ischemia was performed by clamping both common carotid arteries at the level of C3 for approximately 30s. The brain ischemia was ceased by releasing simultaneously both clamps on the left and right common carotid arteries.

Three different types of experiments were performed separately: (1) The effects of vagal stimulation at different intensities to evoke myelinated fiber activation (6.0–6.4 V, 0.1 ms, 10 Hz) or both myelinated and non-myelinated fiber activations (10.5–15.0 V, 0.1 ms, 10 Hz) on the responses of HR before and after administration of hexamethonium (C6, 15 mg/kg, i.v.) were examined in six animals. (2) The changes of HR in response to vagal stimulation to evoke the volley of myelinated fibers (6.7–7.0 V, 0.1 ms, 10 Hz) or the volleys of myelinated and non-myelinated fibers (10.4–15.2 V, 0.1 ms, 10 Hz) were examined before and after intravenous administration of atropine (2 mg/kg) in six animals. (3) In nine rabbits, the effects of C6 on the responses of HR to brain ischemia for approximately 30s were examined. In the cases with an increase in HR seen during brain ischemia in the presence of C6, the effects of propranolol (1 mg/kg, i.v.) were also examined.

The animals that showed a marked reduction in AP after administration of C6
or atropine received an intermittent intravenous infusion (10 ml/(kg·h)) of dextran or Locke's solution.

In each experiment, the brain ischemia and the electrical stimulation of the peripheral end of the cut vagus nerve were continued for approximately 30 s. The maximum decreases of HR seen during vagal efferent stimulation were calculated before and after administration of C₆ or atropine. Changes of HR and MAP in response to brain ischemia were measured at intervals of 5 s. The maximum decreases of HR observed during brain ischemia were calculated before and after administration of C₆ as well as subsequent administration of propranolol in cases with an increase of HR produced by brain ischemia. Statistical analyses were performed by using the multiple paired t-tests, and significance was determined by the \( p < 0.05 \) level. The values of HR and MAP at 0 s after brain ischemia were determined on those obtained after release of clamping both common carotid arteries.

RESULTS

Evoked compound action potentials

Figure 2 shows typical changes in the compound action potentials in evoking a maximal volley in myelinated fibers or volleys in both myelinated and non-myelinated fibers. It shows that electrical stimulation of the left or right vagus nerve produced a compound action potential consisting of a series of volleys with different latencies. The average values of the conduction velocity at the first and second volley were 24.6 ± 1.8 (mean ± SE, \( n = 12 \)) and 13.7 ± 1.1 m/s, respectively. Selective stimulation of those fibers characterized by very rapid conduction velocity

\[
(6.4 \text{ V})
\]

\[
(11.0 \text{ V})
\]

\[
0.2 \text{ mV}
\]

0 10 20 30 40 50 ms

Latency

Fig. 2. The compound action potentials of the left vagus nerve evoked by conventional stimulation at different intensities (6.4 and 11.0 V).
Fig. 3. Effects of vagal stimulation to evoke myelinated fibers (6.4 V, 10 Hz) and both myelinated and non-myelinated fibers (11.0 V, 10 Hz) on the responses of arterial pressure and heart rate before and after administration of hexamethonium (15 mg/kg). ——, period of vagal stimulation.
Fig. 4. Effects of vagal stimulation to evoke myelinated fibers (6.7 V, 10 Hz) and both myelinated and non-myelinated fibers (11.0 V, 10 Hz) on the responses of arterial pressure and heart rate before and after administration of atropine (2 mg/kg). — , period of vagal stimulation.
Table 1. Assessment of the effects of hexamethonium (C₆, 15 mg/kg) and atropine (2 mg/kg) on the bradycardiac responses to the stimulation of either myelinated vagal efferent fibers (A) or myelinated and non-myelinated vagal efferent fibers (B).

<table>
<thead>
<tr>
<th>Vagal stimulation</th>
<th>Bradycardia (beats/min)</th>
<th>Before C₆</th>
<th>After C₆</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: 6.0– 6.4 V, 10 Hz</td>
<td>120.6±6.1</td>
<td>2.4±1.3*</td>
<td></td>
</tr>
<tr>
<td>B: 10.5–15.0 V, 10 Hz</td>
<td>156.6±6.5</td>
<td>37.8±5.7*</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Vagal stimulation</th>
<th>Bradycardia (beats/min)</th>
<th>Before atropine</th>
<th>After atropine</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: 6.7– 7.0 V, 10 Hz</td>
<td>123.8±6.3</td>
<td>2.6±1.4*</td>
<td></td>
</tr>
<tr>
<td>B: 10.4–15.2 V, 10 Hz</td>
<td>158.5±7.4</td>
<td>2.8±1.5*</td>
<td></td>
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</table>

The values are mean±SE (n = 6). *Statistical significance of difference (p < 0.05).

had no effect on heart rate (HR). Recruitment of the third volley, conducting at 5.4±0.4 m/s and reflecting the activity of thinly myelinated fibers, caused a bradycardia. Vagal stimulation at higher stimulus intensity evoked additionally a volley with a slow conduction velocity of 0.9±0.3 m/s, originating from the activity of non-myelinated fibers. When repetitively applied (10 Hz), such stimulation provoked a larger bradycardia. In all of the tested 12 animals it was possible to distinguish a maximal volley in myelinated fibers (conduction velocity >2.5 m/s) from a volley in non-myelinated fibers (conduction velocity <2.5 m/s).

**Vagal stimulation on heart rate**

Figure 3 shows typical changes of arterial pressure (AP) and HR in response to vagal stimulation at different stimulus intensities to evoke activation of either myelinated fibers (6.4 V, 10 Hz) or both myelinated and non-myelinated fibers (11.0 V, 10 Hz) in the presence and absence of hexamethonium (C₆). Selective stimulation of the myelinated vagal fibers produced a bradycardia reducing control HR from 278.6±6.9 (mean±SE, n = 6) to 159.4±5.1 beats/min. When the stimulus intensity was increased, the activation of both myelinated and non-myelinated fibers led to a further decrease in HR to 123.2±6.3 beats/min. After administration of C₆ (15 mg, i.v.), the bradycardia seen during selective stimulation of myelinated fibers disappeared. A small bradycardia reducing HR from 257.4±5.9 to 219.4±6.4 beats/min, when a supramaximal stimulus was applied to the vagus nerve, was still present after C₆-treatment. The summarized results are shown in Table 1.

After intravenous injection of atropine (2 mg/kg, i.v.), vagal stimuli at different intensities did not cause any significant change in HR in all of the tested animals after the treatment with atropine (Fig. 4 and Table 1).
Fig. 5. Effects of brain ischemia on the responses of tracheal side-pressure ($P_{trach}$), arterial pressure, and heart rate before and after administration of hexamethonium (15 mg/kg). ——, period of brain ischemia.
Brain ischemia on heart rate

Figure 5 illustrates typical responses of respiration, AP and HR to brain ischemia for approximately 30s before and after administration of C₆ (15 mg/kg, i.v.). The brain ischemia caused a marked bradycardia and the response was associated with an increase in AP and apnea. After release of brain ischemia the bradycardic response was still observed, and, at the same time, respiration was remarkably augmented. These changes returned to the control levels within 4 min. The bradycardia evoked by brain ischemia was eliminated by C₆-treatment, but there was a small bradycardia after release of brain ischemia. The responses of HR and mean arterial pressure (MAP) to brain ischemia before and after C₆ in 9 rabbits are summarized in Fig. 6. However, hexamethonium treatment significantly reduced the pressor response observed 5–15 s after the onset of brain ischemia. The maximum values of MAP during brain ischemia before and after the treatment with C₆ were 149.6 ± 8.8 and 141.8 ± 6.2 mmHg, respectively, and their respective values were not different from each other.

As illustrated in Fig. 7, after intravenous injection of C₆ an increase in HR occurred during brain ischemia in some rabbits. This tachycardia was greatly diminished by intravenous administration of 1 mg/kg of propranolol. Similar responses were observed in 5 rabbits. The results are summarized in Table 2.

DISCUSSION

The cardiac branches of the vagus nerve are known to contain two groups of fibers, myelinated and non-myelinated. Thus, the question arises as to which type or both types of these two different fiber groups contributes to the bradycardic response to brain ischemia. The purpose of the present study was to elucidate this question.

In this study, the bradycardic evoked by the activation of myelinated vagal efferent fibers, which was blocked following C₆ administration, was smaller than that during the stimulation of an intensity to activate both myelinated and non-myelinated vagal efferent fibers. The latter effect revealed a small bradycardia following C₆ administration and, after such vagal stimulation, also showed a slow return to control. However, vagal stimuli at two different intensities did not cause any significant change in heart rate (HR) in rabbits after administration of atropine. When we consider these results, taken together, it can be suggested that the actions of myelinated and non-myelinated vagal efferent fibers on HR is due to the release of acetylcholine (ACh) and, particularly, the latter action is probably mediated by release of some transmitter which causes the delayed and prolonged release of ACh. The results obtained are in agreement with those of Ford and McWilliam [11] and Wolley et al. [12] in the rabbit, who reported that C₆ eliminated the effect of myelinated fibers on HR but did not influence significantly the fall in HR produced by recruitment of non-myelinated fibers. Although the chronotropic effects of electrical vagal stimulation toward the heart have been
Fig. 6. Changes of heart rate and mean arterial pressure in response to brain ischemia before and after administration of hexamethonium (C₆, 15 mg/kg). Vertical bars are mean ± SE (n = 9). □, before C₆ and ■, after C₆. *Statistical significance of difference (p < 0.05).
Fig. 7. Effects of brain ischemia on the responses of tracheal side-pressure ($P_{trach}$), arterial pressure, and heart rate before and after propranolol (1 mg/kg) in a rabbit after administration of hexamethonium (15 mg/kg). ——, period of brain ischemia.
Table 2. Assessment of the effect of propranolol (1 mg/kg) on the tachycardia produced by brain ischemia in the presence of hexamethonium (15 mg/kg).

<table>
<thead>
<tr>
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<th>Before propranolol</th>
<th>After propranolol</th>
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</thead>
<tbody>
<tr>
<td>Tachycardia (beats/min)</td>
<td>58.4±9.2</td>
<td>9.8±3.5</td>
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</table>

The values are mean ± SE (n = 5).

studied in many species [12-17], there are species differences on the role of non-myelinated vagal efferent fibers in the responses of bradycardia. For example, in cats and dogs the stimulation of non-myelinated fibers has no action on heart rate (HR) [13, 15, 18]. Recruitment of those fibers in rats and guinea-pigs has an additional effect for the bradycardia produced by myelinated fibers and the cardioinhibitory action of non-myelinated fibers are blocked by administration of Cₘ [14, 19]. In the rabbit, we obtained the results that Cₘ was a useful drug to differentiate the bradycardiac response to either myelinated or non-myelinated vagal efferent fiber activation. Accordingly, the effect of Cₘ on the bradycardia evoked by vagal stimulation is a true reflection of the action of myelinated vagal efferent fibers. On the other hand, administration of Cₘ could not block the bradycardia evoked by the activation of non-myelinated fibers in the vagus nerve. This probably implies that Cₘ has no significant effect on the conduction of preganglionic non-myelinated vagal efferent fibers as well as on the non-cholinergic receptors of postganglionic non-myelinated vagal afferent fibers via the axon reflex.

On the other hand, electrical stimulation of the vagus nerve toward the heart in rabbits with chronic supranodal vagotomy produces no action on HR [20]. However, in the cat the majority of the sympathetic cardioaccelerator fibers are located in the vagus nerve below the middle cervical ganglion [21]. In this study, the stimulation applied to the cervical vagus nerve did not cause an increase in HR in the presence of atropine. Thus, it is clear that vagal stimulation used in this study does not alter the HR response via a sympathetic effect.

Although brain ischemia invariably induced remarkable bradycardia, administration of Cₘ eliminated the response. The results obtained suggest that the reflex bradycardia induced by brain ischemia occurs as a result of the activation of myelinated efferent fibers in the vagus nerve. In 4 out of the 9 tested animals, they did not show any significant increase in HR. Since Cₘ is known to block nicotinic transmission in autonomic ganglia, this type of responses may be due to a complete interruption of vagal outflow through nicotinic synapses within the vagal intracardiac ganglia [21, 22]. However, in the remaining 5 animals there was a small but significant increase in HR, and these responses were greatly diminished by administration of propranolol. Similar responses are already reported by the study of Dampney et al. [4], who used the same brain ischemia technique in vagotomized rabbits. As an explanation, the cardioacceleration observed during brain ischemia in the presence of Cₘ may be to the result of either incomplete interruption of vagal

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outflow or insufficient activation of the afferent input from the aortic nerve. Concerning the latter effect, it can be suggested that the magnitude of the pressor response seen during the initial period of brain ischemia is significantly reduced by administration of C6, as compared to that before the treatment. Thus, we cannot rule out the possibility that incomplete blockade of brain ischemia-induced bradycardia in the presence of C6 is partly explained by the depressor response of this ganglion-blocking drug, particularly at the initial period of brain ischemia. The cardioaccelerator response of brain ischemia in rabbits after C0-treatment does not occur as a result of the tonic excitatory effect of the circulating catecholamines because the tachycardia observed during brain ischemia in vagotomized rabbits is not abolished by adrenalectomy [4].

Pressor response that occurs during brain ischemia stimulates the activity of aortic baroreceptors [8]. This probably implies that the activation of aortic baroreceptor reflex mechanism exerts an inhibitory effect on the pressor response evoked by brain ischemia. Indeed, many investigators have clearly demonstrated that electrical stimulation of the aortic nerve results in a depressant effect on the cardiovascular response [23–25]. However, there are consistent differences of the cardiovascular responses to aortic nerve stimulation and brain ischemia. In fact, the brain ischemia-induced pressor response due to the constriction of arterioles in peripheral vascular beds [4, 6] is not significantly altered by surgical denervation of the aortic nerves [7, 8]. Thus, it can be assumed that the rise of MAP resulting from an increase in peripheral resistance produced by brain ischemia is not significantly altered by the depressor effect due to aortic baroreceptor activation. According to the study of Dampney et al. [4], the vasoconstriction in various vascular beds is significantly reduced by sympathetic denervation but some residual vasoconstriction, which is still observed after sympathectomy, is totally abolished by bilateral adrenalectomy. In the present study, C0-treatment significantly reduced the magnitude of the pressor response evoked by brain ischemia at the initial period. This effect would be due to a C0-dependent sympathetic inhibition to the vascular beds. However, administration of C6 did not affect the peak pressor response observed 30s after the onset of brain ischemia, suggesting that, during brain ischemia, the maximum effect of vasoconstriction which occurred in various vascular beds is not significantly affected by administration of ganglion blockers. This finding probably implies that the activation of C0-resistant sympathetic mechanisms such as those mediated by the antidromic excitation of visceral sensory axons contributes to the pressor response remained in the presence of C6 [26, 27].

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