Role of Opioids and GABA in Nucleus Ambiguus in Cardiovascular Inhibitory Responses during Electroacupuncture

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Abstract

Stimulation of cardiopulmonary receptors with phenylbiguanide (PBG) elicits cardiovascular reflex responses, including decreases in blood pressure and heart rate mediated by the brainstem parasympathetic cardiac neurons in the nucleus ambiguous (NA). The present study examined the NA mechanisms underlying the influence of electroacupuncture (EA) on the PBG-induced hypotension and bradycardia. We hypothesized that somatic stimulation during EA modulates PBG responses through opioid and γ-aminobutyric acid (GABA) mechanisms in the NA. Anaesthetized and ventilated rats were studied during repeated stimulation with PBG or cervical vagus while low frequency EA (2 Hz) was applied at PS-6 overlying the median nerve for 30 min and NA neuronal activity, heart rate and blood pressure were recorded. Microinjection of kainic acid into the NA attenuated the PBG-induced bradycardia from 67±17 to 46±14 bpm. EA likewise reduced the PBG-induced depressor and bradycardia reflex by 39 and 68%, respectively. Cervical vagus evoked activity in the NA was reduced rapidly by EA for at least 40 min. Blockade of opioid or GABA receptors using naloxone and gabazine reversed the EA-related modulation of the evoked vagal activity by 73 and 53%, respectively. Thus, EA at PS-6 decreases PBG evoked hypotension and bradycardia as well as the evoked NA activity through opioid and GABA systems. Supported by HL-63313.

Introduction

Electroacupuncture (EA) reduces reflexly induced increases in blood pressure in animals (1). We have shown in a series of studies that several neurotransmitters, including opioids, serotonin and γ-aminobutyric acid (GABA) are important during the inhibitory effects of acupuncture (2; 3; 6). The rostral ventrolateral medullary nucleus ambiguus (VRN), which is characterized by bradyphrenia, hypotension and vasodilation (4). The decrease in heart rate as a result from cardiac parasympathetic inhibition involves the medullary nucleus ambiguous (5). Modulation of the neuronal activity in nucleus ambiguous may therefore reduce blood pressure.

Previous study has suggested that EA also may be able to modify decreases in blood pressure. In this regard, acupuncture partially reverses the hypotensive response to hemorhage (5). This suggests that EA also may be able to modulate reflexly evoked decreases in blood pressure. We therefore hypothesized that EA reduces the bradycardia and decrease in blood pressure occurring in response to stimulation of the Bezold-Jarisch reflex. We further hypothesized that opioids and GABA in the nucleus ambiguous are involved in the processing of this cardiovascular inhibitory reflex during EA.

Materials and Methods

• Cats anesthetized with ketamine and xylazine
• Intubation and cannulation

A. Cardiovascular Responses

• Craniootomy to expose medulla for microinjection into nucleus ambiguous
• PBG iv administration for induction of cardiovascular reflex (Bezold Jarisch) response
• Acupuncture needle placement at PS-5 overlying median nerve for treatment

B. Modulatory Neuronal Responses

• Isolation of vagal preganglionic neurons for antidromic stimulation
(2 Hz, 0.5 ms, < 0.4 mA) of nucleus ambiguous
• Craniootomy, respectively, to exposure medulla into nucleus ambiguous and extracellular recording of these neurons
• Acupuncture needle placement at PS-5 overlying median nerve (2 Hz, 0.5 ms, 2-4 mA) for characterization

The Bezold-Jarisch reflex is reduced by 30 min stimulation of EA at acupoints PS-5,6, in part, through its action in the nucleus ambiguous. GABA and opioids in the nucleus ambiguous are involved in processing of EA mediated reflex response with respect to cardiovascular reflex inhibition mediated through premotor cardiac vagal preganglionic neurons.

Protocols

Cardiovascular Responses

1. Repeated 5-HT3-receptor stimulation with PBG (400 μg/kg every 10 min)
   • microinjection of kainic acid (1 mM, 50 nl) into nucleus ambiguous

2. Repeated PBG application in presence of: 30 min stimulation of electroacupuncture
   • microinjection of gabazine (27 mM) or naloxone (100 nM)

Medullary Responses

1. Characterization of ambiguus neuron
   • antidromic stimulation
   • responses to cardiac vagal and median nerves stimulation
   • determinations of time and frequency domain analyses

2. Repeated vagal cardiac nerve stimulation every 10 min to evoke excitatory parasympathetic premotor cardiovascular ambiguous activity

3. Repeated vagal cardiac nerve stimulation following: 30 min stimulation of electroacupuncture
   • microinjection of gabazine
   • microinjection of naloxone

Summary

• Repeated stimulation with PBG every 10 min caused consistent reflex depressor and bradycardia responses.
• Neurons in the nucleus ambiguous were characterized by their projection to the heart, cardiac rhythmicity, and baroreactivity.
• Stimulation of the cardiac vagal nerve every 10 min evoked repeatable neuronal responses in nucleus ambiguous.
• Stimulation of EA for 30 min at the PS-5,6 acupoints, overlying the median nerve, reduced the cardiac vagal reflex responses evoked by stimulation of cardiac pulmonary afferents as well as nucleus ambiguous neuronal activity for prolonged periods of time (400±20 min).
• Microinjection of kainic acid to block activity in the nucleus ambiguous transiently inhibited the vagal excitatory and sympathomimetic cardiovascular responses to PBG.
• Blockade of either opioid or GABA receptors in the nucleus ambiguous disabled the effects of EA on the cardiovascular and neuronal responses.
• However, opioid or GABA receptor blockade in the nucleus ambiguous did not alter the neuronal responses to stimulation of cardiac vagal nerve.

Conclusion

The Bezold-Jarisch reflex is reduced by 30 min stimulation of EA at acupoints PS-5,6, in part, through its action in the nucleus ambiguous. GABA and opioids in the nucleus ambiguous are involved in the processing of EA mediated reflex response with respect to cardiovascular reflex inhibition mediated through premotor cardiac vagal preganglionic neurons.

Figure 1. Decreases in blood pressure and heart rate were reduced by electroacupuncture (EA). Panels A and B are control data showing consistent blood pressure and heart rate responses to repeated cardiopulmonary 5-HT3 receptor stimulation with PBG every 10 min. Panels C and D display the effects of EA on these vagal excitatory and sympathomimetic cardiovascular reflex responses.

Figure 2. Bar histograms display decreases blood pressure and heart rate in response to administration of PBG is before and after microinjection of kainic acid in nucleus ambiguous. Inactivation of these neurons reduced the bradycardia (*P<0.05) transiently to 32 ± 3%, thus confirming the role of the nucleus ambiguous in the reflex chronotropic response.

Figure 3. Bar histograms display neuronal activity in the nucleus ambiguous during repeated stimulation of the cardiac vagal afferents every 10 min. Panel A shows consistent evoked responses. Panel B shows reduced activity during and after stimulation to PS-5,6 for 30 min. * indicates decreased evoked discharge rate compared with the elicited activity prior to stimulation of EA, P< 0.05.

Figure 4. Cardiac vagal evoked activity in the nucleus ambiguous reduced during EA that involves GABA and opioid receptors. Neither antinominal gabazine nor naloxone influenced the PBG induced evoked responses (Panels A1 and B1). On the other hand, blockade of both GABA and opioid receptors during the inhibitory effects of EA (the discharge frequency) abolished acupuncure action on the neuronal activity.

Figure 5. Prominent parasympathoexcitatory cardiac neuron in the nucleus ambiguous (NA) receiving median nerve (MN) and cardiac vagal convergent input that also displayed baroreactivity and rhythmicity with the cardiac cycle. Panels A1 and A2 are representative neurograms of the median and vagal nerve evoked activation following stimulation artifacts. The neuron displayed cardiac rhythmicity with a coherence of 0.81 at a frequency of 4.2 Hz (Panel B) determined with frequency domain analysis. Pulse triggered averaging (Panel C) analyzed with time domain analysis also showed the cardiovascular rhythmicity. Baroreactivity determined with phenylephrine (PE) to elevate blood pressure increased the discharge frequency (Panel D). The neuron was located in the NA projecting to the heart was characterized by collision testing through antidromic stimulation of the cardiac vagal branch. (Panels E and F) indicate the time of stimulation and stimulation artifact of the MN or vagal nerve, respectively.

References

1. Choo SM, Shiue LI, Tjen-A-Looi SC, Li P, and Longhurst JC. The Bezold-Jarisch reflex is reduced by 30 min stimulation of EA at acupoints PS-5,6, in part, through its action in the nucleus ambiguous. GABA and opioids in the nucleus ambiguous are involved in the processing of EA mediated reflex response with respect to cardiovascular reflex inhibition mediated through premotor cardiac vagal preganglionic neurons.


