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Dexmedetomidine decreases inhibitory but not excitatory neurotransmission to cardiac vagal neurons in the nucleus ambiguus

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Abstract

Dexmedetomidine, an α_2 adrenergic agonist, is a useful sedative but can also cause significant bradycardia. This decrease in heart rate may be due to decreased central sympathetic output as well as increased parasympathetic output from brainstem cardiac vagal neurons. In this study, using whole cell voltage clamp methodology, the actions of dexmedetomidine on excitatory glutamatergic and inhibitory GABAergic and glycinergic neurotransmission to parasympathetic cardiac vagal neurons in the rat nucleus ambiguus was determined. The results indicate that dexmedetomidine decreases both GABAergic and glycinergic inhibitory input to cardiac vagal neurons, with no significant effect on excitatory input. These results provide a mechanism for dexmedetomidine induced bradycardia and has implications for the management of this potentially harmful side effect.

1. Introduction

Dexmedetomidine is a highly specific α_2 adrenergic agonist increasingly popular as an anesthetic adjunct and sedative agent. α_2 Adrenergic agonists have unique properties including a mimicry of a natural sleep state with easy arousability [3]. Other properties, such as analgesia, and a lack of respiratory depression, have further increased interest in the clinical use of dexmedetomidine. The most significant adverse effects with dexmedetomidine use have been cardiovascular in nature involving both hypotension and bradycardia. The incidence of bradycardia is described as occurring in 9–42% of patients [1,11]. There are several possible reasons for this bradycardia including decreased central sympathetic output, decreased catecholamine release, and an increased central

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parasympathetic output, and it is possible more than one of these mechanisms may be involved. Most current hypotheses have focused on decreased central sympathetic output [4,7].

The primary regulation of heart rate in a sedated state, however, arises from the parasympathetic nervous system, specifically from brainstem cardiac vagal neurons. Clonidine, a less specific α_2 adrenergic agonist than dexmedetomidine, has been shown to increase the excitability of cardiac vagal neurons via decreased inhibitory neurotransmission [10] suggesting a likely similar target for the effects of dexmedetomidine on cardiac vagal neurons. The purpose of this study is to test if synaptic neurotransmission to cardiac vagal neurons are altered by clinically relevant concentrations of dexmedetomidine. Cardiac vagal neuron activity is determined by the summation of synaptic activity from excitatory glutamatergic and inhibitory GABAergic and glycinergic neurotransmission to these neurons. In this study these excitatory and inhibitory synaptic inputs were isolated to determine the effects of dexmedetomidine on each synaptic input. Characterization of the targets of action of dexmedetomidine in the neurotransmission to cardiac vagal neurons will identify mechanism(s) for the bradycardia that often occurs with dexmedetomidine.

2. Results

Dexmedetomidine dose dependently decreased spontaneous GABAergic and glycinergic inhibitory post synaptic currents (IPSCs) in cardiac vagal neurons. The frequency of spontaneous GABAergic events was inhibited from an average control of 9.8 ± 2.2 Hz to 4.1 ± 1.2 Hz and 3.1 ± 0.8 Hz in the presence of dexmedetomidine at concentrations of 8nM and 10nM, respectively ($p < 0.05$, figure 1). Inhibitory postsynaptic glycinergic currents were likewise inhibited by dexmedetomidine in a dose dependent fashion. Administration of dexmedetomidine suppressed the frequency of glycinergic IPSCs in cardiac vagal neurons from an average control of 10.8 ± 1.9 Hz to 5.3 ± 1.5 Hz ($n=8$, $P < 0.05$) and 3.7 ± 0.8 Hz ($n=8$, $P < 0.01$) at doses of 8nM and 10nM, respectively (figure 2). Dexmedetomidine did not alter the amplitude of inhibitory GABAergic and glycinergic IPSCs or the holding current in cardiac vagal neurons. In contrast spontaneous glutamatergic excitatory post synaptic currents were not altered at any concentrations up to 10 nM dexmedetomidine ($n=8$ in each group, data not shown). To test whether the dexmedetomidine-induced inhibitory effect on GABAergic and glycinergic neurotransmission to cardiac vagal neurons is mediated by α_2 noradrenergic receptors, we applied yohimbine, an α_2 antagonist, before and during dexmedetomidine administration. The application of yohimbine (2 μ M) alone increased spontaneous GABAergic inputs but not glycinergic neurotransmission to cardiac vagal neurons (figure 3). Yohimbine abolished the dexmedetomidine-induced inhibitory response of both GABAergic and glycinergic IPSC's in cardiac vagal neurons (figure 3).

3. Discussion

Taken together, these results in this study indicate that dexmedetomidine selectively decreases inhibitory input to cardiac vagal neurons, via activation of α_2 receptors, with little effect on excitatory input. This investigation provides a potential mechanism for the heart rate and autonomic effects of dexmedetomidine. Decreasing inhibitory neurotransmission to

cardiac vagal neurons would result in an increase in excitability of parasympathetic neurons that project to the heart and subsequent bradycardia. Increased parasympathetic activity to the heart has potential relevance to the etiology and therapy for dexmedetomidine induced bradycardia.

This work did not find any evidence that dexmedetomidine alters the glutamatergic neurotransmission to cardiac vagal neurons. However previous work has shown dexmedetomidine can inhibit the release of glutamate upon activation of $\alpha(2A)$ adrenoceptors on cerebrocortical nerve terminals, and this effect is likely due to inhibition of presynaptic voltage-dependent $Ca(2+)$ channels [2]. Additionally, dexmedetomidine decreases evoked glutamate release from hippocampal neurons upon increases in extracellular potassium, and hypoxic stress [13]. However another study has shown dexmedetomidine does not alter excitatory amino acid release in rabbits upon transient global ischemia [8]. These differences, the lack of effect of dexmedetomidine on excitatory neurotransmission to cardiac vagal neurons, while dexmedetomidine alters excitatory neurotransmission to other neurons, are likely due to differences in the channels and receptors in presynaptic glutamatergic terminals that surround these different neurons. Additionally, in this study, yohimbine, an $\alpha 2$ antagonist, increased spontaneous GABAergic, but not glycinergic, neurotransmission to cardiac vagal neurons. This would suggest that, in this preparation, $\alpha 2$ adrenergic receptors are endogenously active and inhibit GABAergic neurotransmission to CVNs, but are not present, or endogenously active, in glycinergic pathways to CVNs.

In general there is a decrease in parasympathetic control of heart rate with aging [12]. Pediatric populations would therefore be at a potentially higher risk for dexmedetomidine induced bradycardia if the parasympathetic pathway has a significant role. Indeed younger infants have a greater incidence of dexmedetomidine induced bradycardia than older children [9]. Also children can be extremely sensitive to anticholinergic therapy for α_2 adrenergic agonist related bradycardia [9].

One important further avenue for research in this area involves identifying the source and modulation of adrenergic neurotransmission to neurons involved in cardiovascular homeostasis. One likely source of catecholaminergic input to the brainstem cardiorespiratory network is from the locus coeruleus, a catecholaminergic brain region essential for wakefulness and vigilance. Recent studies using optogenetics and light stimulation has found photoactivation of catecholaminergic locus coeruleus neurons augments inhibitory neurotransmission to cardiac vagal neurons [14]. In addition, there is evidence of extensive arborization of presympathetic neurons onto CVNs originating from the ventral brainstem, a region which contains many catecholaminergic neurons [5]. It is possible that specific α_2 agonists like dexmedetomidine unmask the strong inhibitory input on cardiac vagal neurons originating from the locus coeruleus and/or pre-sympathetic neurons in the ventral brainstem.

In previous work the bradycardic effects of dexmedetomidine has been commonly ascribed to a decreased central sympathetic output. Dexmedetomidine has also been shown to depress sinus and AV nodal function [6]. However this work indicates it is also likely that

parasympathetic activity is a target of dexmedetomidine and the resulting increased activity of central cardiac vagal output plays an important role. The clinical responses to anticholinergic therapy and the lack of response to this therapy in the denervated transplant patient supports this mechanism [15]. In summary, the results of this study reveal potential mechanisms for the increased parasympathetic activity to the heart, and decreased heart rate, during dexmedetomidine administration.

4. Experimental Procedure

All animal procedures were performed with the approval of the Animal Care and Use Committee of The George Washington University in accordance with the recommendations of the panel on euthanasia of the American Veterinary Medical Association and the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

4.1 Labeling of CVNs in the nucleus ambiguus

Cardiac vagal neurons in the nucleus ambiguus were identified by the presence of a retrograde fluorescent tracer. Briefly, Sprague–Dawley rat pups (postnatal days 1–7) were anesthetized and exposed to hypothermia to slow the heart. The heart was exposed by a right thoracotomy and the retrograde fluorescent tracer X-rhodamine-5-(and 6)-isothiocyanate (XRITC) was injected into the fat pads at the base of the heart. Parasympathetic cardio-inhibitory neurons in the nucleus ambiguus were later identified in-vitro by the presence of the fluorescent tracer.

4.2 Brainstem slice preparation, identification, and recording

Pups were anesthetized with isoflurane 2–5 days post-injection and sacrificed by cervical dislocation. The hindbrain was rapidly removed and placed in cold physiological saline solution composed of the following (in mM): 140 NaCl, 5 KCl, 2 CaCl₂, 5 glucose, 10 Hepes, bubbled with 100% O₂, pH 7.4. Slices (700 µm) were cut in a horizontal orientation. The horizontal medullary slice preparation retained the local network pathways and CVNs. The tissue was placed in a recording chamber and perfused (4 ml min⁻¹) with artificial cerebrospinal fluid containing (in mM): 125 NaCl, 3 KCl, 2 CaCl₂, 26 NaHCO₃, 5 glucose, 5 HEPES, equilibrated with 95% O₂–5% CO₂, pH 7.4. Slices were viewed with infrared illumination and differential interference optics. Neurons containing fluorescent XRITC tracer were identified by superimposing the fluorescence and infrared images on a video monitor.

4.3 Whole-cell patch clamp and electrophysiological recording

Patch pipettes (2.5–4.5 MΩ) were visually guided to the surface of individual CVNs using differential interference optics and infrared illumination. In voltage-clamp experiments examining excitatory synaptic events pipettes were filled with a solution containing 130 mM potassium gluconate, 10 mM HEPES, 10 mM EGTA, 1 mM CaCl₂, and 1 mM MgCl₂. In voltage-clamp experiments examining inhibitory synaptic events pipettes were filled with a solution containing 150 mM KCl, 4 mM MgCl₂, 10 mM EGTA, 2 mM Na-ATP and 10 mM HEPES. Voltage clamp recordings were made with Axopatch 200B and pCLAMP 8 software (Axon Instruments, Union City, CA, USA). All synaptic activity in

parasympathetic cardioinhibitory neurons were recorded at -80 mV. Only one experiment was performed in each slice of tissue. Drugs were applied by inclusion in the perfusate.

4.4 Isolation of excitatory or inhibitory responses

Glutamatergic excitatory synaptic events were isolated by bath applying the GABA_A receptor antagonist gabazine ($25\mu\text{M}$) and the glycinergic receptor antagonist strychnine ($1\mu\text{M}$). GABAergic inhibitory responses were isolated by adding strychnine, with the non NMDA antagonist CNQX ($50\mu\text{M}$) and the NMDA receptor antagonist AP5 ($50\mu\text{M}$). Glycinergic inhibitory responses were isolated by applying CNQX, AP5 and gabazine. Yohimbine ($2\mu\text{M}$), an α_2 antagonist, was bath applied 5 minutes prior to dexmedetomidine at a concentration of 8nM . The threshold for the glutamatergic, GABAergic and glycinergic synaptic events was set at five times the root mean square of background noise and was detected using MiniAnalysis software.

Data analyses—All electrophysiological data were digitized and collected via Clampex (10.2) and analyzed using Clampfit (10.2). Synaptic events were detected using MiniAnalysis version 5.6.12 (Synaptosoft, Decatur, GA). All data are presented as mean $\pm$ SEM. Statistical analysis was performed using GraphPad Prism 5 software. Student's t-test, paired t-test and one-way ANOVA with a post hoc Dunnett's test were performed where indicated. Significant difference was set at $P < 0.05$.

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Figure 1. Spontaneous GABAergic IPSCs in CVNs

Dexmedetomidine dose dependently decreased spontaneous GABAergic neurotransmission to cardiac vagal neurons.

Dexmedetomidine, at concentrations of 8 nM and 10 nM, significantly inhibited the frequency of gamma-aminobutyric acid (GABA) mediated inhibitory postsynaptic currents (IPSCs) in parasympathetic cardiac vagal neurons. Representative traces of GABAergic IPSCs in CVNs are shown at the top, at dexmedetomidine concentrations of 1, 5, 8, and 10 nM ($n=7$, except $n=5$ in recovery). The statistic results in each group are illustrated in the bottom histogram. In this and all subsequent figures, * denotes $P<0.05$, and ** denotes $P<0.01$.

Figure 2. Spontaneous glycinergic IPSCs in CVNs

Dexmedetomidine suppressed spontaneous glycinergic inputs to CVNs.

Dexmedetomidine significantly inhibited glycinergic IPSCs in parasympathetic cardiac vagal neurons at concentrations of 8 and 10 nM. The effect of dexmedetomidine at doses of 1, 5, 8, and 10 nM on glycinergic IPSCs in a typical cardiac vagal neuron is shown in the top traces, and the summary data from eight cardiac vagal neurons are shown in the bottom histogram.

Figure 3. Effect of yohimbine on inhibitory GABAergic and glycinergic IPSCs

The α_2 antagonist, yohimbine (2 μ M), abolished dexmedetomidine-induced inhibitory effects on both GABAergic and glycinergic IPSC's in cardiac vagal neurons

Yohimbine (2 μ M) alone augmented GABAergic but not glycinergic IPSCs in CVNs (n=7 and 8, respectively). Yohimbine blocked dexmedetomidine-induced inhibitory effect on GABAergic and glycinergic IPSCs in CVNs (n=7 and 8, respectively) when applying yohimbine (2 μ M) and dexmedetomidine (8 nM) together.