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β ADRENERGIC RECEPTOR MODULATION OF NEUROTRANSMISSION TO CARDIAC VAGAL NEURONS IN THE NUCLEUS AMBIGUUS

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Abstract—β-adrenergic receptors are a class of G protein-coupled receptors that have essential roles in regulating heart rate, blood pressure, and other cardiorespiratory functions. Although the role of β adrenergic receptors in the peripheral nervous system is well characterized, very little is known about their role in the central nervous system despite being localized in many brain regions involved in autonomic activity and regulation. Since parasympathetic activity to the heart is dominated by cardiac vagal neurons (CVNs) originating in the nucleus ambiguus (NA), β adrenergic receptors localized in the NA represent a potential target for modulating cardiac vagal activity and heart rate. This study tests the hypothesis that activation of β adrenergic receptors alters the membrane properties and synaptic neurotransmission to CVNs. CVNs were identified in brainstem slices, and membrane properties and synaptic events were recorded using the whole-cell voltage-clamp technique. The nonselective β agonist isoproterenol significantly decreased inhibitory GABAergic and glycinergic as well as excitatory glutamatergic neurotransmission to CVNs. In addition, the β_1-selective receptor agonist dobutamine, but not β_2 or β_3 receptor agonists, significantly decreased inhibitory GABAergic and glycinergic and excitatory glutamatergic neurotransmission to CVNs. These decreases in neurotransmission to CVNs persisted in the presence of tetrodotoxin (TTX). These results provide a mechanism by which activation of adrenergic receptors in the brainstem can alter parasympathetic activity to the heart. Likely physiological roles for this adrenergic receptor activation are coordination of parasympathetic-sympathetic activity and β receptor-mediated increases in heart rate upon arousal.

Key words: parasympathetic, adrenergic, ambiguus, vagal.

Adrenergic receptors (ARs) are a diverse class of G protein-coupled receptors involved in the regulation of heart rate, blood pressure, as well as metabolic function (Zheng et al., 2004, 2005). Three subclasses of βARs have been identified, each having distinct and often opposing effects dependent upon the targeted tissue and receptor activated. For example, activation of β_1 receptors in the heart by endogenous catecholamine release from postganglionic sympathetic neurons leads to increased heart rate, contractility, and cardiac output, making these receptors an excellent clinical target for treating arrhythmia, tachycardia, and hypertension (Esler, 2000). Activation of β_2 receptors causes dilation of blood vessels and relaxation of the respiratory tract, advantageous for increasing blood flow to the exercising muscle and enhancing ventilation (Nials et al., 1993). Although the β_3 subclass has traditionally been considered to regulate metabolic functions such as lipolysis, recent work has suggested β_3 receptor activation in the heart also causes a decrease in contractility by altering Na^+-K^+ pumps (Bundgaard et al., 2010).

Despite the powerful and endogenous activation of βARs in the peripheral nervous system and the frequent use of βAR antagonists used to treat cardiovascular diseases such as cardiac arrhythmias and hypertension, very little is known about the role of βARs within the central nervous system and, in particular, the modulation of parasympathetic activity, which dominates the control of heart rate in the adult rat. Parasympathetic activity originates from cardiac vagal neurons (CVNs) located in the nucleus ambiguus (NA) (Mendelowitz and Kunze, 1991; Mendelowitz, 1999). Although the activity of αARs have been well characterized in CVNs, the binding of catecholamines to either α or β ARs can elicit very different physiological responses (Philbin et al., 2010; Boychuk et al., 2011). Additionally, previous studies have identified βARs both in proximity to, and in populations of, neurons known to project to CVNs, therefore making βARs a potential brainstem target likely to alter neurotransmission to CVNs. For example, immunohistochemical studies have identified dense β_1AR localization in the NA, nucleus of the solitary tract, ventrolateral medulla, as well as other brainstem structures involved in cardiorespiratory function (Paschalis et al., 2009). Additionally, autoradiographical and immunohistochemical techniques indicate the presence of β_2 receptors in proximity to the NA (Ampatzis and Dermon, 2010). Furthermore, specific hybridization studies of brain mRNA indicate the presence of β_3 receptors in the brainstem, although at much lower levels than in areas such as the hippocampus and cortex (Summers et al., 1995). The aim of this study was to test whether βARs are endogenously active and can modulate three essential inputs to CVNs as follows: the inhibitory GABAergic and glycinergic and excitatory glutamatergic neurotransmission to CVNs in the NA, as well as evoke direct postsynaptic changes in CVNs.

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Abbreviations: AR, adrenergic receptor; CVN, cardiac vagal neuron; EPSCs, excitatory postsynaptic currents; IPSCs, inhibitory postsynaptic currents; LC, locus coeruleus; mEPSCs, miniature EPSCs; mIPSCs, miniature IPSCs; NA, nucleus ambiguus; RVLM, rostral ventrolateral medulla; TTX, tetrodotoxin.

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EXPERIMENTAL PROCEDURES

Procedures

All animal procedures were performed in compliance with the institutional guidelines at The George Washington University and were in accordance with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association and the NIH publication (85–23, revised 1996) "Guide for the Care and Use of Laboratory Animals." The minimal number of animals was used and attention was given to minimize any possible discomfort.

In an initial surgery, 2–5-day-old Sprague–Dawley rats were anesthetized with hypothermia to slow the heart rate and aid in recovery. A right thoracotomy was performed to expose the heart and the retrograde tracer, rhodamine (XRITC, Invitrogen, Carlsbad, CA, USA, 2% solution, 20–50 μl), was then injected into the pericardial sac to retrogradely label CVNs.

On the day of the experiment, 1–3 days after the injection of the fluorescent tracer, the animal was anesthetized with isoflurane and sacrificed by cervical dislocation. The brain was rapidly removed and immersed in a cold HEPES buffer (4 °C) with the following composition: NaCl (140 mM), KCl (5 mM), $CaCl_2$ (2 mM), glucose (5 mM), and HEPES (10 mM). The buffer was continuously oxygenated with 100% O_2. Using a dissection microscope, the hindbrain was isolated. The brain was glued to a stage and placed in the slicing chamber of a vibratome filled with the aforementioned buffer solution. Slices 500–600 μm in thickness were cut. The slices were then mounted in a perfusion chamber and submerged in a perfusate with the following composition: NaCl (125 mM), KCl (3 mM), $CaCl_2$ (2 mM), $NaHCO_3$ (26 mM), glucose (5 mM), and HEPES (5 mM) and oxygenated with 95% O_2/5% CO_2 gas mixture. The osmolarity of all solutions was 285–290 mOsm, and the pH was maintained between 7.35 and 7.4.

Electrophysiological recording

Individual CVNs located in the NA were identified by the presence of the fluorescent tracer rhodamine, and differential interference contrast optics along with infrared illumination and infrared-sensitive video detection cameras were used to gain better spatial resolution. The pipettes were filled with a solution consisting of KCl (150 mM), $MgCl_2$ (4 mM), EGTA (10 mM), Na-ATP (2 mM), and HEPES (10 mM) at a pH of 7.3 for recording inhibitory GABAergic and glycinergic events and K-gluconic acid (150 mM), HEPES (10 mM), EGTA (10 mM), $MgCl_2$ (1 mM), and $CaCl_2$ (1 mM) at a pH of 7.3 for recording excitatory glutamatergic events. CVNs were studied by whole-cell patch-clamp techniques and were voltage clamped at a holding potential of −80 mV.

GABAergic inhibitory postsynaptic currents (IPSCs) were isolated by adding strychnine (1 μM), a glycinergic receptor antagonist, to the perfusate. To isolate glycinergic IPSCs, gabazine (25 μM), a $GABA_A$ receptor antagonist, was included in the perfusate. Glutamatergic excitatory postsynaptic currents (EPSCs) were isolated by adding gabazine (25 μM) and strychnine (1 μM) to the perfusate.

The following pharmacological agents were applied by inclusion in the perfusate after a 5–10-min control period: the nonselective β agonist isoproterenol (100 μM), the β_1 selective agonist dobutamine (10 μM), the β_2 selective agonist albuterol (10 μM), and the β_3 selective agonist BRL 37344 (1 μM). Each agent was applied exclusively to a slice, and no slice was used for more than one experiment. In another set of experiments, atenolol (100 μM), a β_1 selective antagonist, was applied in the perfusate for 5 min before and during application of dobutamine (10 μM) for 5 min while isolating for inhibitory or excitatory synaptic events. In a different set of experiments, increasing concentrations of dobutamine were applied in the perfusate for 5-min intervals after isolating for GABAergic or glycinergic inhibitory events or excitatory glutamatergic events. The concentrations of dobutamine applied in order were 0.01, 0.1, 1, 10, and 100 μM, with each concentration being applied immediately after 5-min application of the previous concentration. In another set of experiments, tetrodotoxin (TTX), a voltage-gated sodium channel blocker, was applied in the perfusate 5 min before and during application of dobutamine (10 μM) for 5 min while isolating for inhibitory or excitatory synaptic events. All drug concentrations were selected based on selective activation of the targeted receptor based on previous work in the literature.

The effect of isoproterenol (100 μM) on membrane resistance was also examined. A voltage-step from −80 mV to −85 mV was applied to assess changes in the postsynaptic membrane resistance in CVNs.

Isoproterenol was purchased from Tocris Bioscience (Ellisville, MO, USA). TTX was purchased from Ascent Scientific (Princeton, NJ, USA). All other drugs were purchased from Sigma (St. Louis, MO, USA). MiniAnalysis (Synaptosoft version 4.3.1) was used to analyze experimental traces. The threshold for the GABAergic, glycinergic, and glutamatergic events was five times the root mean square of noise. Membrane resistance was determined using ClampFit (Molecular Devices). The last 3 min of recorded synaptic events before drug application were averaged for control values. A 2-min transition period was then allowed after application of drugs before averaging the next 3 min of synaptic events for changes during receptor activation. Results are presented as mean±SE and statistically compared with a paired Student *t*-test except for the experiments with atenolol and repeated dobutamine applications in which an analysis of variance (ANOVA) with repeated measures was performed (for a significant difference of * $P<0.05$).

RESULTS

Application of the nonselective β agonist isoproterenol (100 μM) evoked a significant decrease in both inhibitory GABAergic and glycinergic neurotransmission to CVNs, as well as excitatory glutamatergic neurotransmission to CVNs, see Fig. 1. GABAergic IPSC frequency significantly decreased from 7.5±0.7 Hz to 4.9±0.6 Hz ($n=7$, * $P<0.05$) as did glycinergic IPSC frequency, which decreased from 6.9±0.6 Hz to 5.8±0.8 Hz ($n=10$, * $P<0.05$) in the presence of isoproterenol. Similarly, glutamatergic excitatory postsynaptic current (EPSC) frequency to CVNs decreased from 6.5±1.1 Hz to 3.6±0.8 Hz in the presence of isoproterenol, see Fig. 1 ($n=8$, * $P<0.05$). To test whether isoproterenol was affecting membrane resistance in postsynaptic neurons, a series of voltage steps were applied. Isoproterenol did not elicit any significant change in resistance in CVNs (control=605±124 MΩ, isoproterenol=633±283 MΩ; $n=6$, $P>0.05$).

To determine which subtype of βARs was responsible for inhibiting neurotransmission to CVNs, βAR subtype selective agonists were applied in additional experiments. The β_1 selective agonist dobutamine (10 μM) elicited a significant decrease in both inhibitory GABAergic and glycinergic neurotransmission to CVNs and excitatory glutamatergic neurotransmission to CVNs, see Fig. 2. In the presence of dobutamine, GABAergic IPSC frequency decreased from 6.8±0.8 Hz to 4.7±0.6 Hz ($n=8$, * $P<0.05$) and glycinergic IPSC frequency decreased from 7.3±1.0 Hz to 5.8±0.9 Hz ($n=8$, * $P<0.05$). Glutamatergic EPSC frequency decreased from 6.1±1.1 Hz to 4.3±0.9 Hz ($n=7$, * $P<0.05$). In addition, dobutamine significantly decreased the amplitude of GABAergic and glycinergic