α-Adrenoceptor blockade modifies neurally induced atrial arrhythmias

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Richer LP, Vinet A, Kus T, Cardinal R, Ardell JL, Armour JA. α-Adrenoceptor blockade modifies neurally induced atrial arrhythmias. Am J Physiol Regul Integr Comp Physiol 295: R1175–R1180, 2008. First published August 20, 2008; doi:10.1152/ajpregu.00840.2007.—Our objective was to determine whether neurally induced atrial arrhythmias can be modified by α-adrenergic receptor blockade. In 30 anesthetized dogs, trains of five electrical stimuli (1 mA; 1 ms) were delivered immediately after the P wave of the ECG to mediastinal nerves associated with the superior vena cava. Regional atrial electrical events were monitored with 191 atrial unipolar electrodes. Mediastinal nerve sites were identified that reproducibly initiated atrial arrhythmias. These sites were then restimulated following 1 h (time control, n = 6), or the intravenous administration of naftopidil (α1-adrenergic blocker: 0.2 mg/kg, n = 6), yohimbine (α2-adrenergic blocker: 1 mg/kg, n = 6) or both (n = 8). A ganglionic blocker (hexamethonium: 1 mg/kg) was tested in four dogs. Stimulation of mediastinal nerves sites consistently elicited atrial tachyarrhythmias. Repeat stimulation after 1 h in the time-control group exerted a 19% decrease of the sites still able to induce atrial tachyarrhythmias. Hexamethonium inactivated 78% of the previously active sites. Combined α-adrenoceptor blockade inactivated 72% of the previously active sites. Bradycardia responses induced by mediastinal nerve stimulation were blunted by hexamethonium, but not by α1-adrenergic blocker. Naftopidil or yohimbine alone eliminated atrial arrhythmia induction from 31% and 34% of the sites (similar to time control). We conclude that heterogeneous activation of the intrinsic cardiac nervous system results in atrial arrhythmias that involve intrinsic cardiac neuronal α-adrenoceptors. In contrast to the global suppression exerted by hexamethonium, we conclude that α-adrenoceptor blockade targets intrinsic cardiac local circuit neurons involved in arrhythmia formation and not the flow-through efferent projections of the cardiac nervous system.

neuromodulation; cardiac nervous system; ganglionic blockade

MATERIALS AND METHODS

Animals. A total of 30 adult mongrel canines (either sex), weighing 15–40 kg, were used in this study. Experiments were performed in accordance with guidelines for animal experimentation (World Medical Association-American Physiological Society, 2002) and approved by the institutional animal care committee of the University of Montreal. Animals were anesthetized with sodium thiopental (25 mg/kg iv, supplemented as required), intubated and maintained under positive-pressure ventilation. After all surgical procedures had been completed, the anesthetic agent was changed to α-chloralose (50 mg/kg iv bolus, supplemented with 25 mg/kg iv as required).

Surgical preparation. Following a transthoracic incision, the pericardium was incised to expose the heart. Left ventricular and aortic pressures (Millar electronic pressure sensors) and a lead II ECG were recorded on a rectilinear pen recorder (Nihon Kohden, Tokyo, Japan). Atrioventricular blockade was induced by formaldehyde injection (35%; 0.1 ml) into the AV node to isolate atrial from ventricular electrical events. Right ventricular pacing (60 beats/min) was instituted to maintain adequate cardiac output.

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Atrial epicardial mapping. Multiple silicone plaques carrying 191 unipolar recording electrodes (4.6–5.9 mm spacing) were positioned on the ventral, lateral, and dorsal surfaces of the right and left atrium (7). The unipolar leads and a lead II ECG were connected to a multichannel recording system (EDI 12/256, Institut de Génie Biomédical, École Polytechnique de Montréal) controlled by custom-made software (Cardiomap III: www.crhis.umontreal.ca/cardiomap) using a PC. The 191 unipolar electrograms (measured with reference to Wilson’s central terminal derived from the four limb leads) were amplified by programmable-gain analog amplifiers (0.05–450 Hz) and converted to digital format at 1,000 samples/s-channel⁻¹. Data were stored on hard disk and retrieved for detailed analysis.

Electrical stimulation of mediastinal nerves. Atrial tachyarrhythmias were induced in each animal by delivering electrical stimuli to individual right-sided mediastinal nerves that course over the ventral and ventrolateral surfaces of the caudal-most part of the superior vena, as identified by their accompanying vessels (6). Active sites were identified that, when stimulated electrically, immediately induced atrial arrhythmias. The most frequently identified response consisted of a bradycardia followed by an episode of spontaneous and self-terminating atrial tachyarrhythmia/fibrillation (7). Once identified, each active locus was marked with ink for repeated stimulations. Electrical stimuli were applied to neuronal elements located on the intrapericardial portion of the superior vena cava on 1) the first 1–2 cm cranial to its junction with the right atrium and 2) the first 1–2 cm caudal to that same anatomical landmark. Electrical stimuli were delivered focally via a bipolar electrode (1.5-mm spacing) mounted on a roving probe that was connected to a battery-driven current source controlled by a programmable stimulator (Bloom Associates, Philadelphia, PA). Trains of 5 electrical stimuli (1 mA, 1 ms duration; 5-ms pulse interval) were delivered during the refractory period of the closest atrial regions (beginning 30 ms after excitation of a reference electrode). This was done to avoid atrial muscle capture. Stimulations were interrupted immediately after the onset of tachycardia prior to AF. If no sites were identified at 1 mA, the intensity of the stimulation was increased gradually to 1.5 and 2 mA such that 3–5 active neural sites could be identified prior to repetition (time-control group) or pharmacological treatment. Following the administration of drugs, if a previously identified active site no longer induced AF at the control stimulation intensity, the intensity was increased to 2 mA.

Experimental design. After identifying 3–5 active right-sided mediastinal nerve sites, animals were randomized to one of five treatment groups. Pharmacological agents were administered within 5 min of completing active site identification. Group 1 (n = 6) evaluated the effects of time. In this group, active sites identified at baseline were restimulated 1 and 2 h later. Group 2 consisted of four dogs whose mediastinal nerves were stimulated before and after administering the ganglionic blocker hexamethonium (1 mg/kg iv). In Group 3 (n = 8), the α1-adrenergic blocking agent naftopidil (0.2 mg/kg iv) and α2-adrenergic blocking agent yohimbine (1 mg/kg iv) were administered in combination, and the active sites restimulated with the ganglionic blocker hexamethonium, atrial cycle length prolongation from 366 to 494 ms), followed by rapid transition to atrial fibrillation (disorganized beats), atrial flutter (organized beats without a pause between them), atrial tachycardia (organized beat with a pause between beats), or sinus rhythm.

Data collected prior to and after drug administration were subjected to paired t-test analysis and chi-squared testing. Comparisons between experimental groups were made using univariate (for individual variables) and multivariate two-way ANOVA. The level of certainty for rejecting the null hypothesis was P ≤ 0.05. Data are presented as means ± SD.

RESULTS

Select mediastinal nerve response characteristics. Among the 30 dogs, a total of 150 active mediastinal neuronal projection sites were identified adjacent to the superior vena cava that, when stimulated, consistently elicited atrial tachyarrhythmias. A typical response consisted of an immediate bradycardia that reached its maximum (17% change) within 1.3 ± 0.7 s of the initial stimulus application, followed by a spontaneous premature atrial depolarization initiating a tachyarrhythmia that terminated spontaneously within seconds to minutes (median duration of 8.7 s; min = 0.3 s; max = 2,158 s) of stimulus cessation (Fig. 1). The majority of the tachyarrhythmias were the atrial tachyarrhythmias: latency (defined as the interval from the first applied stimulus train to tachyarrhythmia initiation); tachyarrhythmia (sinus tachycardia) duration; tachyarrhythmia interbeat intervals; and duration of atrial flutter/fibrillation. For each animal, characteristics of atrial bradycardia and/or tachyarrhythmias were averaged from the multiple mediastinal nerve stimulation sites. For post hoc analysis, data were also subgrouped based on efficacy of treatment(s) to extinguish (or not) neurally induced tachyarrhythmias. Induced-rhythm types were classified based on information obtained from the biaxial epicardial recording plaques. Classes included atrial fibrillation (disorganized beats), atrial flutter (organized beats without a pause between them), atrial tachycardia (organized beat with a pause between beats), or sinus rhythm.

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Fig. 1. Atrial arrhythmia induction in response to electrical stimuli applied to a mediastinal nerve. Atrial recordings derived from a unipolar electrode on the ventral right atrial free wall shows dissociated atrial and ventricular (v) complexes in a canine preparation with atrioventricular block. Intermittent application of bursts of electrical stimuli (1 mAmp, 1-ms duration, 5-ms pulse interval) to a cranial right-sided mediastinal nerve (arrows) during the atrial refractory period in control states (A) typically evoked a bradycardia (cycle length prolongation from 366 to 494 ms), followed by rapid transition to atrial fibrillation (here, atrial fibrillation) that self-terminated after 3.3 s. Similar responses to repeat stimulation were induced in control states. Following nicotinic ganglionic blockade (B; hexamethonium), atrial cycle length increased to 477 ms, with a residual, but blunted, bradycardia being induced when electrical stimuli were applied to the same mediastinal nerve site. In this state, atrial tachyarrhythmia did not occur, even when the stimuli were applied for prolonged periods of time (21 s). Numbers in bold represent atrial cycle lengths.
classified as atrial fibrillation (AF) or atrial flutter (AFl) with early epicardial breakthroughs primarily localized to the sinoatrial nodal pacemaker complex, right atrial appendage or Bachmann’s Bundle. No atrial response was elicited when the electrode was moved to immediately adjacent non-neural sites, even at the highest stimulation intensity tested (2 mA).

Time control (group 1, n = 6 animals). To assess the temporal stability of bradycardia and tachyarrhythmia induction, a total of 28 active neural sites were subjected to repeat stimulation at hourly intervals for 2 h in 6 separate animals. Throughout this period, sinus rate remained stable and bradycardias were reproducibly induced at all neural sites (Fig. 2A, left). Averaged across animals, after each subsequent hour, AF or AFl was no longer inducible from ~20% of the previously responsive sites in spite of the fact that the magnitude of the induced bradycardias was similar (Fig. 2A, right). Increasing the intensity of the electrical stimuli at the unresponsive sites did not restore arrhythmia induction. Consequently, all subsequent treatment protocols (groups 2–5) were performed within 1 h of identifying active neural sites.

Hexamethonium (group 2, n = 4 animals). Hexamethonium increased basal atrial cycle lengths (Fig. 2B, left). It also blunted the initial bradycardias elicited by mediastinal nerve stimulation (Fig. 2B, left). Comparing sites where hexamethonium eliminated atrial fibrillation/flutter induction to those in which it was maintained, there was no difference in induced changes in basal heart rate or the residual bradycardia response elicited by nerve stimulation (Fig. 2B, right).

In control states, the principal mediastinal nerve-induced arrhythmias were atrial tachyarrhythmias or flutter (Fig. 3, control) with early epicardial breakthroughs located primarily to the SA nodal pacemaker complex, right atrial appendage or Bachmann’s bundle (Fig. 4, control). Following hexamethonium, mediastinal nerve-induced tachydysrhythmias were eliminated from 78% of the previously active sites (Fig. 1B; Fig. 3, hexameth.). Pacemaker activity remained localized primarily within the SA nodal pacemaker complex (Fig. 4, hexameth.). For residual tachyarrhythmias, latency (+46%) and duration (−85%) were altered after hexamethonium.

Combined α1&2-adrenoceptor blockade (group 3, n = 8 animals). Averaged across animals, the number of mediastinal nerve sites at which tachyarrhythmias were induced by electrical stimulation was reduced by 72 ± 15% following the combined administration of naftopidil and yohimbine (Fig. 5 and Fig. 3, bottom). This was significantly greater than that identified in time controls (19 ± 11%). The elimination of mediastinal-induced tachyarrhythmias by combined adrenergic blockade was accompanied by less dispersion of the primary pacemaker location compared with control conditions (Fig. 4, α-block sensitive). For the stimulation sites that maintained tachyarrhythmias postcombined alpha blockade (Fig. 3, α-block resistant), the rhythm type and early epicardial breakthrough points remained similar to control (Fig. 4, α-block resistant). For all sites, there was no significant difference in the magnitude of mediastinal nerve induced bradycardia when comparing rhythms before to after combined alpha blockade (Fig. 2, bottom). Moreover, there was no difference in tachyarrhythmia latencies, cycle lengths, or durations under control conditions, among the AF/AFl- and AF/AFl+ subgroups, nor between responses for those sites with residual tachyarrhythmias postblockade (Fig. 6). Arterial blood pressure was reduced by combined alpha blockade (systolic/diastolic pressure, mmHg; control 138 ± 30 / 93 ± 21; α1&2-adrenoceptor blockade 111 ± 22/76 ± 8; P < 0.05). Heart rate was unaffected by combined alpha blockade (151 ± 23 beats/min, control; 143 ± 25 beat/min, α1&2-adrenoceptor blockade; P = 0.48).

Single α-adrenoceptor subtype blockade (naftopidil, group 4, n = 6 animals, or yohimbine, group 5, n = 6 animals). When administered alone, naftopidil or yohimbine induced a 31% (11/36) or 34% (11/32), respectively, reduction in the number of active neural sites at which atrial tachyarrhythmias were induced by electrical stimulation. Such reductions were not significantly different from the 19 ± 11% average reduction identified in the time control group (group 1).
Previous findings indicated that neuronal imbalances within discrete elements of the intrinsic cardiac nervous system augments the atrial arrhythmogenic substrate (2, 7). They also indicate the possibility of effectively targeting select intrinsic cardiac neuronal populations, in this instance, one possessing alpha-adrenoceptors, to stabilize such imbalance in the suppression of atrial arrhythmia formation. The latter is in accord with the fact that atrial tachyarrhythmias of neural origin can be suppressed by hexamethonium (7).

There are multiple targets of alpha blockade that could potentially impact upon atrial electrical stability, including cardiac-related neurons, cardiomyocytes, and vascular smooth muscle. In that regard, the stabilization of atrial electrical activity occurred, even though heart rate and blood pressure remained close to control values, indicating that overall autonomic neural status and vascular tone was largely unaltered by the blocking doses used herein. With respect to cardiomyocytes, the electrophysiological properties of atrial muscle, including the sinus node, can be directly affected by alpha adrenoceptor blockade, albeit, such effects are minor in nature (9, 26). Because of these considerations and the location of neural elements activated, we propose that any tachyarrhythmia suppression initiated by \( \alpha_1 \) and \( \alpha_2 \)-adrenoceptor blockade involves alpha-adrenergic-mediated neurotransmission within the intrinsic cardiac nervous system. While the effects of single \( \alpha_1 \) or \( \alpha_2 \) alpha blockade by themselves may impact to some degree on the atrial arrhythmogenic potential, the data presented herein suggest that the effects of combined blockade may act in a synergistic manner to exert their influence.

In the past, we have indicated that electrical stimulation of select mediastinal nerve inputs to discrete elements of the intrinsic cardiac nervous system augments the atrial arrhythmogenic substrate (2, 7). They also indicate the possibility of effectively targeting select intrinsic cardiac neuronal populations, in this instance, one possessing alpha-adrenoceptors, to stabilize such imbalance in the suppression of atrial arrhythmia formation. The latter is in accord with the fact that atrial tachyarrhythmias of neural origin can be suppressed by hexamethonium (7).

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In the past, we have indicated that electrical stimulation of select mediastinal nerve inputs to discrete elements of the intrinsic cardiac nervous system can lead to excessive and heterogeneous activation of the intrinsic cardiac nervous system that increases the arrhythmogenic substrate for atrial tachyarrhythmias (7, 19). The suppression of such events by combined \( \alpha_1 \) and \( \alpha_2 \)-adrenoceptor blockade identified in this work may reside in its stabilizing effects on select neuronal elements of the intrinsic cardiac nervous system.

\( \alpha_1 \) and \( \alpha_2 \)-adrenoceptors and autonomic neurons. \( \alpha \)-adrenoceptors have been identified on intrinsic cardiac parasympathetic efferent neurons (29). Activation of \( \alpha_1 \)-adrenoceptors associated with intrinsic cardiac parasympathetic neurons induces a two-step response (16), an initial inhibitory response (18) followed by a longer lasting excitatory phase, in which parasympathetic intrinsic cardiac neurons fire repetitively (15). Although in vitro studies have failed to demonstrate modula-
tion of sympathetic efferent neurons by $\alpha_1$-adrenoceptor agonists (24, 25), select populations of intrinsic cardiac neurons can be activated in situ by locally applied $\alpha_1$- or $\alpha_2$-adrenergic agonists (4). In fact, alpha adrenergic activation of intrinsic cardiac local circuit neurons enhances regional cardiac, electrophysiological, and mechanical function (4). Furthermore, it is known that $\alpha_2$-adrenoceptors on parasympathetic and sympathetic efferent nerve terminals (29) act to inhibit neurotransmitter release (1). Taken together, these data suggest the potential for $\alpha$-adrenoceptor blockade to modify intrinsic cardiac local circuit and efferent neuronal function.

**Hexamethonium vs. combined $\alpha_1$- and $\alpha_2$-adrenoceptor blockade.** Although targeting neural elements within the intrinsic cardiac nervous system with either hexamethonium or $\alpha_{1,2}$-adrenergic blockade was effective in suppressing tachyarrhythmia formation, bradycardia responses were blunted by hexamethonium but not by $\alpha_{1,2}$-adrenergic blockade. In fact, combined $\alpha_1$- and $\alpha_2$-adrenoceptor blockade minimally affected the initial bradycardia induced by mediastinal nerve stimulation, regardless of whether or not subsequent atrial tachyarrhythmias were suppressed (Fig. 2). These data indicate that the primary throughput of parasympathetic efferent neuronal projections was maintained after $\alpha$-adrenoceptor blockade.

Preganglionic and postganglionic neuronal elements within the intrinsic cardiac nervous system play a major role in the induction of atrial tachyarrhythmias when excessively and heterogeneously activated. Targeting of neural elements within the intrinsic cardiac nervous system with systemic hexamethonium or $\alpha_{1,2}$-adrenergic blockade stabilized atrial pacemaker function and location in response discrete stimulation of efferent inputs to its ganglia. In the case of alpha blockade, this occurred without interfering with local autonomic control of chronotropic function (Figs. 1 and 4, bottom). In addition to sympathetic and parasympathetic efferent postganglionic neurons, the intrinsic cardiac nervous system contains local circuit neurons (2). It has been proposed that this population functions as interneurons coordinating intraganglionic and interganglionic neuronal interactions (11). As such, this system represents an interactive network coordinating sympathetic and parasympathetic efferent neuronal outflows at the level of the target organ (4). Some of these local circuit neurons possess $\alpha$-adrenoceptors. As such, $\alpha$-adrenoceptors may be involved not only as feedback mediators at the terminals of efferent postganglionic neurons (30) but also as processors of information analogous to what is observed in the central nervous system (13). Our knowledge of the role that local circuit neurons play in cardiac control remains limited. Data obtained from this study indicate that combined $\alpha_{1,2}$-adrenoceptor blockade may not only target such neurons (4), but also modifies/stabilizes information processing within the intrinsic cardiac nervous system to reduce the neuronal component of the arrhythmogenic substrate. In contrast, hexamethonium exerts a more global suppression, impacting not only local circuit neurons but also neurotransmission in the sympathetic and parasympathetic efferent limbs of the cardiac nervous system.

**Perspectives and Significance**

Current pharmacological or physical (i.e., ablation) management of atrial fibrillation targets atrial myocytes, as well as regional cardiac neural tissue that is concentrated around the pulmonary vein orifices (22, 23). The results of this study indicate that intrinsic cardiac local circuit neurons may also be regarded as a potential therapeutic target in arrhythmia suppression. Notably, pharmacological therapy would act to spare atrial tissue, something that ablation of regional intrinsic cardiac neural tissue does not accomplish (23). Such targeted drug therapy has the potential to stabilize the multiple components within the intrinsic cardiac nervous system, rather than destroying critical elements of a system that are essential for coordinating regional cardiac indices (2, 4). Taken together, these data support the concept that stabilization of the intrinsic cardiac nervous system in the presence of excessive inputs may affect the latter’s involvement in atrial arrhythmia formation.
REFERENCES


