Autonomic Neuroscience: Basic and Clinical 201 (2016) 24–31

Contents lists available at ScienceDirect

Autonomic Neuroscience: Basic and Clinical

journal homepage: www.elsevier.com/locate/autneu

Complex and interacting influences of the autonomic nervous system on cardiac electrophysiology in conscious mice

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ARTICLE INFO

Article history:
Received 15 April 2016
Received in revised form 15 August 2016
Accepted 28 August 2016

Keywords:
Sympathetic
Parasympathetic
Wenckebach cycle length
Sinus node recovery time
Atrio-ventricular interval
Electrocardiogram

ABSTRACT

Mice may now be the preferred animal model for biomedical research due to its anatomical, physiological, and genetic similarity to humans. However, little is known about accentuated antagonism of chronotropic and dromotropic properties in conscious mice. Accordingly, we describe the complex and interacting influence of the autonomic nervous system on cardiac electrophysiology in conscious mice. Specifically, we report the effects of single and combined cardiac autonomic blockade on measurements of pulse interval (heart rate), atrio-ventricular interval, sinus node recovery time (SNRT), SNRT corrected for spontaneous sinus cycle, and Wenckebach cycle length in conscious mice free of the confounding influences of anesthetics and surgical trauma. Autonomic influences were quantified as the change in parameter induced by its selective blocker (Sympathetic or Parasympathetic Effect) or as the difference between the intrinsic value and the value after a selective blocker (Sympathetic or Parasympathetic Tonus). Sympatho-Vagal Balance (SVB) was assessed as the ratio of control interval to intrinsic interval. SVB suggests slight parasympathetic dominance in the control of cardiac electrophysiology intervals. Furthermore, results documents a complex interaction between the sympathetic and parasympathetic divisions of the autonomic nervous system in the control of cardiac electrophysiology parameters. Specifically, the parasympathetic effect was greater than the parasympathetic tonus in the control of cardiac electrophysiology parameters. In contrast, the sympathetic effect was smaller than the sympathetic tonus in the control of cardiac electrophysiology parameters. Results have important implications because actions of pharmacological agents that alter the autonomic control of cardiac electrophysiology are transformed by these interacting mechanisms.

1. Introduction

Mammalian heart rate and cardiac electrophysiology are profoundly influenced by the sympathetic and parasympathetic divisions of the autonomic nervous system. Heart rate is slowed by parasympathetic nervous system activity via the muscarinic M2 receptor (Fisher et al., 2004) and elevated by sympathetic nervous system activity via the beta 1-adrenergic receptor (Rohrer et al., 1998). In addition, the sympathetic and parasympathetic divisions of the autonomic nervous system alter heart rate and cardiac electrophysiology through complex and interacting mechanisms.

Sympathetic stimulation has similar effects on both atrial and ventricular electrophysiology and is pro-arrhythmic for both chambers (Shen and Zipes, 2014; Kapa et al., 2010). In particular, beta-adrenergic receptor stimulation, by increasing intracellular cAMP levels, increases heart rate, atrial-ventricular (A-V) nodal conduction, and contractile force while shortening atrial and ventricular refractoriness. Furthermore, beta-adrenergic stimulation enhances the development of afterdepolarizations and triggered beats (Engel, 1978; Schwartz et al., 1993; Wharton et al., 1992; Zipes, 1991).

In contrast to sympathetic stimulation which has similar effects on atrial and ventricular electrophysiology, parasympathetic stimulation has opposing effects on these chambers. Specifically, parasympathetic stimulation prolongs ventricular action potential duration and the effective refractory period, (Martins and Zipes, 1980; Ng et al., 2001). In contrast, parasympathetic stimulation reduces the atrial effective refractory period (Zipes et al., 1974; Wijffels et al., 1995) while increasing electrophysiological heterogeneity (Fareh et al., 1998) and promoting early afterdepolarization (EAD) (Burashnikov and Antzelevitch, 2003). Accordingly, parasympathetic stimulation is pro-arrhythmic in the atria but antiarrhythmic in the ventricles (Wijffels et al., 1995). Furthermore, parasympathetic activation of muscarinic-cholinergic receptor decreases intracellular cAMP levels, heart rate, AV nodal conduction, and contractile force.

There also exists a complex interaction between the sympathetic and parasympathetic divisions of the autonomic nervous system. Early

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http://dx.doi.org/10.1016/j.autneu.2016.08.017
1566-0702/

pioneering studies documented that the reduction in heart rate produced by parasympathetic activation was greater during sympathetic stimulation (Rosenblueth and Simeone, 1934; Samaan, 1935; Warner and Russell, 1969; Levy and Zieske, 1969a; Stramba-Badiale et al., 1991; Vanhoutte and Levy, 1980). This effect was documented to be due, in part, to the fact that efferent parasympathetic stimulation inhibited efferent sympathetic activation at both pre- and post-junctional sites (Vanhoutte and Levy, 1980; Takahashi and Zipes, 1983; Shen and Zipes, 2014) as well as reduced cAMP levels to markedly influence heart rate, ventricular function, intracellular calcium handling, and cardiac electrophysiology (Levy and Zieske, 1969b; Brack et al., 2004; Martins and Zipes, 1980; Shen and Zipes, 2014). Thus, parasympathetic effects became progressively stronger with increasing sympathetic activity. Furthermore, sympathetic effects are substantially smaller in the presence of high parasympathetic activity. This complex interaction has been called accentuated antagonism (Levy and Zieske, 1969b) and *suggests* that changes in cardiac electrophysiology resulting from changes in sympathetic control cannot be interpreted accurately unless concurrent parasympathetic activity is taken into account. Similarly changes in cardiac electrophysiology resulting from changes in parasympathetic activity cannot be interpreted accurately unless concurrent sympathetic activity is taken into account (Rosenblueth and Simeone, 1934; Samaan, 1935; Warner and Russell, 1969; Levy and Zieske, 1969a).

In addition to activation of the parasympathetic and sympathetic divisions, the complex and interacting influences on the autonomic nervous system on cardiovascular function can also be studied indirectly by using pharmacological cardiac autonomic blockade (Sayin et al., 2016; Chen et al., 1995a). Results obtained from these studies have been analyzed by a variety of approaches. Comparisons have been made among parasympathetic and sympathetic effects, as well as parasympathetic and sympathetic tonus. A parasympathetic effect is defined as the response to cardiac muscarinic cholinergic receptor blockade (difference between control value and the value after muscarinic cholinergic blockade). A sympathetic effect is defined as the response to cardiac beta1-adrenergic receptor blockade (difference between the control value and the value after beta1-adrenergic receptor blockade). It has been *suggested* that these effects are difficult to interpret because it is challenging to distinguish the direct result of blockade from the indirect result (Gava et al., 1995; Negrão et al., 1992; Chen and DiCarlo, 1997). For example, the heart rate after muscarinic cholinergic receptor blockade (parasympathetic effect) is the result of the direct effect of removal of the parasympathetic influence on the heart as well as the indirect effect of the unopposed sympathetic influence on the heart in response to blockade of the parasympathetic limb. Another potential limitation when using the parasympathetic (or sympathetic) effect is that a possible change in intrinsic heart rate is not considered. Any change in intrinsic heart rate would affect the final heart rate.

In an attempt to reduce the influence of these two suggested limitations, investigators have used parasympathetic and sympathetic tonus (Gava et al., 1995; Negrão et al., 1992; Chen and DiCarlo, 1997; Sayin et al., 2016). Parasympathetic tonus is defined as the difference between the intrinsic value and the value after beta1-adrenergic receptor blockade. Sympathetic tonus is defined as the difference between the intrinsic value and the value after muscarinic cholinergic receptor blockade. Thus, both parasympathetic and sympathetic tonus represent the effect of the parasympathetic and sympathetic nervous systems on the heart without the influence of the opposing limb of the autonomic nervous system. By using sympathetic and parasympathetic tonus, investigators are also able to account for any potential change in intrinsic heart rate. However, it is important to note that no consensus exists on the use of these two approaches (Sayin et al., 2016).

This complex interaction between the sympathetic and parasympathetic systems has important implications because actions of pharmacological agents that alter the autonomic nervous system control of cardiac electrophysiology are transformed by these interacting mechanisms (Morady et al., 1988; Mirro et al., 1980). Specifically, agents used in the treatment of cardiovascular disorders have varying effects depending on background levels of autonomic nervous system functioning (Fukudo et al., 1992; Mirro et al., 1980; Hayano et al., 1990). Furthermore, the interacting influences must also be considered in the context of stress and exercise because the high sympathetic activity associated with these conditions is modified by parasympathetic activity (Morady et al., 1988; Mirro et al., 1980).

To address these concepts, we describe for the first time, the complex and interacting effects of the autonomic nervous system on heart rate and cardiac electrophysiology in a conscious, murine model of cardiac electrophysiology (Lujan and DiCarlo, 2014). The mouse has significant advantages over other experimental models for the investigation of autonomic control of cardiac electrophysiology (Bryda, 2013). The mouse is readily available, inexpensive, has a high throughput, and gives the investigator the ability to create genetically modified models. As a result, conscious mice have replaced many of the other animals, such as dogs, cats and rats in biomedical research because of the many advantages (Bryda, 2013; Lujan et al., 2012a, 2012b; Lujan and DiCarlo, 2013; Lujan and DiCarlo, 2014). However, virtually nothing is known regarding autonomic control of cardiac electrophysiology in conscious mice. Furthermore, when considering accentuated antagonism, investigators must distinguish between chronotropic and dromotropic properties to fully understand cardiac function because each property has its own distinctive relationship with the two divisions of the autonomic nervous system.

Accordingly, using two analytical approaches we investigated the autonomic control of pulse interval (heart rate), atrio-ventricular (AV) interval, sinus node recovery time (SNRT), SNRT corrected for spontaneous sinus cycle (cSNRT), and Wenckebach cycle length (WCL) in a conscious murine model free of the confounding influences of anesthetics and surgical trauma. The approach allows for the accurate documentation of the complex and interacting influence of the autonomic nervous system on cardiac electrophysiology in conscious mice and may be adopted for advancing the concepts and ideas that drive autonomic research.

2. Methods

2.1. Experimental subject

All surgical and experimental procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee and conformed to the American Physiological Society Guiding Principles in the Care and Use of Animals. Studies determining the complex and interacting influences of the autonomic nervous system on cardiac electrophysiology parameters were conducted in 8 male C57BL/6J mice (15 weeks of age), a strain commonly used in transgenic studies (Berul et al., 1996).

2.2. Surgical procedures

2.2.1. Instrumentation

All surgical procedures were performed using aseptic surgical measures. Adult, male C57BL/6 mice were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and supplemental doses (10–20 mg/kg, i.p.) were administered if the mice regained the blink reflex or responded during the surgical procedures.

The hearts were approached via a left thoracotomy through the second intercostal space. Teflon coated stainless steel wire electrodes (0.003 in., part no. 316 SS 7/44T, Medwire, Mount Vernon, NY) were sutured 1–2 mm apart with 8.0 silk on the surface of the left atrial appendix as previously described in rats (Rodenbaugh et al., 2003a; Rodenbaugh et al., 2003b) and mice (Lujan and DiCarlo, 2014). The stimulating wires were tunneled subcutaneously and exteriorized at the back of the neck. Subsequently, a catheter from a telemetry device