Nerve Growth Factor Mediates the Vious Cycle between Hyperactivity of Ganglionated Plexus and Atrial Fibrillation

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Abstract

Ganglionated Plexus (GP) is a complex neural network composed by intrinsic cardiac autonomic nervous system (ANS) and is mainly located in fat pads around the antrum of the pulmonary veins (PVs). Recent studies demonstrated hyperactivity of GPs and atrial fibrillation (AF) formed a vicious cycle, to be specific, hyperactivity of the cardiac GPs facilitated the initiation and maintenance of AF and the activity of cardiac GPs increased as AF continued. In addition, research has confirmed that the Nav1.8 channel is highly expressed in GPs and is closely related to activity of GPs and the inducibility of AF. Nerve growth factor (NGF) is an important neurotrophic factor and the expression of NGF in GPs is up-regulated during AF over time, which could trigger the release of SP in the heart *via* TRPV1 signaling pathways. Besides, SP could rapidly increase the activity of the Nav1.8 channel, demonstrating the increment of Sensory nerve action potentials. Therefore, we hypothesized that up-regulated NGF during AF could increase the activity of GPs through TRPV1-SP-Nav1.8 channel pathways and contributes to stability of AF. If this hypothesis is proved to be correct, future studies based on this link may help to find new therapeutic targets for the treatment of AF.

Keywords: Ganglionated Plexus; Atrial fibrillation; Nerve growth factor; Nav1.8 channel; TRPV1 receptor

Short Communication

Atrial fibrillation (AF) is the most common cardiac arrhythmia, and the prevalence of AF is expected to increase dramatically over the next few decades [1]. Although AF itself is not typically lethal, it is associated with an increased risk of stroke, heart failure, and dementia, as well as cardiovascular-related and all-cause mortality [2]. In addition, AF accounts for more than one-third of all arrhythmia-related hospitalizations [3]. Once AF is initiated, it is inclined to sustain itself and cause changes in progressive electrical remodeling [4] and structural remodeling [5] of the atria and that promote the occurrence and maintenance of AF, knowing as the concept of "AF begets AF". Recent years, emerging evidence indicated that autonomic remodeling has a close link with the initiation and maintenance of AF [6].

The Relationship between Atrial Neural Hyperactivity and AF Inducibility

Cardiac autonomic nerve is made up of two main components: the extrinsic and intrinsic autonomic nervous system (ANS). The former consists mainly of ganglia and their axons located outside the heart and the latter is composed mainly of ganglionated plexi and their axons, which are typically embedded in the epicardial fat pads [7]. As to human hearts, there are at least 7 GP and 4 major left atrial GP are located around the antrum of the PVs [8]. In addition, most of intrinsic cardiac neurons in GPs were found to be cholinergic.

Recently, Several lines of evidence suggested that autonomic remodeling plays an important role in the pathogenesis of AF, which mainly demonstrated as the hyperactivity of the cardiac autonomic nervous system (ANS) [6,9,10] and sympathetic hyperinnervation [11] and changes of several protein such as nerve growth factor (NGF), small conductance calcium-activated potassium channel type 2 (SK2), neurturin (NRTN) [12,13], to be specific, researchers found that both of extrinsic cardiac nerve activity (ECNA) and intrinsic cardiac nerve activity (ICNA) increased as AF continued. Moreover, in a canine model, researchers found that Stimulation of either the Aortic Root GP or anterior right ganglionated plexuses (GP) could trigger the initiation of AF [14,15]. On the contrary, several studies found that 1) destruction of epicardial fat pads by radiofrequency ablation or surgical excision [16,17], 2) blockade of autonomic nerve within GPs could induced a progressive increase in the AF threshold and prevented the initiation and maintenance of AF [18]. Taken together, these facts confirmed that hyperactivity of the cardiac ANS and AF formed a vicious cycle and suppressed the activity of GPs could effective inhibit the inducibility of AF.

The Expression of NGF within Gps Up-Regulated as AF Continued

NGF is one of the most extensively studied neurotrophic factors, which can be synthesized by several types of cells, including lymphocytes, fibroblasts, macrophages and mast cells [19], and it is vital for the survival, differentiation, and synaptic activity of the sympathetic and sensory nervous systems [20,21]. There is considerable evidence that the expression of NGF became increasingly up-regulated throughout the progress of AF [12,13], high-frequency electrical field stimulation (HFES) of both parasympathetic [22] and sympatheticneurons *in vitro* to mimic rapid atrial depolarization further indicated that the cardiac autonomic neurons are an important source of NGF. Besides, abundant studies have shown that NGF plays an essential role in hyperalgesia and inflammatory pain [23], which are mainly associated with Nav1.8 channels [24] and TRPV1 receptors [25]. To be specific, acute exposure of NGF can enhance the activity of TRPV1 receptor within half an hour through phosphoinositide-3-kinase and mitogen activated protein kinase signaling pathways [26] and subsequently trigger the

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release of SP, which could rapidly potentiates Nav1.8 sodium current *via* PKCε-dependent signaling pathway [27]. On the other hand, chronic exposure of NGF has been shown to lead to an increase in the expression level of the Nav1.8 channel *via* transcriptional modification mechanisms [23,28].

Nav1.8 Channel, TRPV1 Receptor and SP are Co-Expressed in Cardiac Vagal Neurons within Gps

Nav1.8 (encoded by SCN10A) is a TTX-R Na+ channel [24], which is localized predominantly in small/medium nociceptive C/Aδ-type dorsal root ganglia (DRG) neurons [25], and plays a critical role in the upstroke of action potential in neurons. Recent studies showed that the Nav1.8 channel rather than other types of Voltage-gated sodium channels (VGSCs) is highly expressed within GPs [29,30]. Besides, blockade of the Nav1.8 channel by its special chemical antagonists A803467 could significantly inhibit the activity of GPs [31,32] and counteract the cholinergic effects of GPs stimulation, indicating that the Nav1.8 channel is highly expressed in cardiac vagal neurons and is closely related to the activity of GPs.

The capsaicin receptor TRPV1 (transient receptor potentialvanilloid-1, previously known as vanilloid receptor subtype 1) is a nonselective cation channel, which can be activated by physical and chemical mediators including noxious heat, protons, vanilloid compounds [33]. In recent studies, it has been confirmed that TRPV1 receptors are expressed in cardiac afferent neurons in the nodose ganglia (NG). Besides, Immunocytochemistry further revealed that Substance P (SP), as a member of tachykinin family, also expressed in the cardiac vagal afferent neurons in the NG [34], it was reported that during ischemia/reperfusion injury, TRPV1 receptors played an important role in mediating cardiac ischemic preconditioning *via* increasing endogenous SP and led to improved cardiac performance in the diabetic heart [35,36]. In addition, it has reported that SP could activate NK-1 receptor and potentiate Nav1.8 sodium current *via* PKCε-dependent signaling pathway [27].

Hypothesis

Considering the background above, we hypothesized that NGF was a crucial factor contributing to the vicious cycle between GPs hyperactivity and AF. This hypothesis is supported by three main lines of evidence. First, in a model of AF, direct neural recordings of the activity of GPs increased as AF progressed [12,37], which in turn maintained short and dispersed atrial effective refractory periods to sustain AF. On the contrary, inhibition of the activity of GPs by physical or chemical methods can potently suppress the initiation and maintenance of AF. Second, there is considerable evidence that the expression of NGF became increasingly up-regulated throughout the progress of AF and cells experiment further confirmed that cardiac autonomic nerve is an important resource of NGF. Third, Immunocytochemistry revealed that TRPV1 channel, SP and the Nav1.8 channel definitely expressed in the cardiac vagal afferent neurons, and NGF can not only rapidly enhance the activity of Nav1.8 channel through stimulation of TRPV1 receptor but also increase the expression of Nav1.8 channel.

In summary, the expression of NGF up-regulated as AF continued and induced the hyperactivity of GPs through TRPV1-SP-Nav1.8 channel pathways. The resulting hyperactivity of GPs and AF form a vicious cycle and reciprocally enhance each other.

Evaluation and Discussion of the Hypothesis

To test our hypothesis, several mongrel dogs will be enrolled and Experiments will follow the guidelines outlined by the Care and Use of Laboratory Animals of the National Institutes of Health. First, heart needs to be exprosured by means of thoracotomy and neurons within GPs need to be isolated, the expression of TPRV1 receptor, SP and Nav1.8 channel should be tested by western blot and Quantitative RT-PCR to verify they are indeed co-expressed within GPs. Second, the activity of GPs needs to be recorded by BL-420E multi-lead physiological recorder in baseline and in the present of NGF with or not K252a (a high-affinity nerve growth factor receptor blocker) [38] respectively. The location of GPs can be confirmed by high frequency stimulation (HFS; 20 Hz, 10–150 V, 1–10 ms pulse width) where GPs are presumed to be located. As most of intrinsic cardiac neurons in GPs were found to be cholinergic, HFS will induce a significant parasympathetic response, when mean R-R interval demonstrate as ≥ 50% increase, this location is assigned as a GP site [39]. Furthermore, atrial effective refractory period, and the cumulative window of vulnerability should be tested to verify the relationship between the activity of GPs and the inducibility of AF.

The second step is to confirm the mechanism that why NGF can induce the hyperactivity of GPs, in order to answer this question, we will apply an antagonist or agonist to inhibit or mimic the biological effect of NGF, TRPV1 receptor, SP respectively to examine their influence on the activity of GPs and the inducibility of AF, the methods above can refer to these articles [27,31,32,40].

We predict that the Nav1.8 channel, TRPV1, SP are indeed expressed within GPs, and up-regulated NGF within GPs promotes activity of GPs through TRPV1 receptor-SP-Nav1.8 channel pathways and leads to the vicious cycle between GPs hyperactivity and AF.

Underlying Clinical Perspective

Atrial fibrillation (AF) is a complex arrhythmia, although intensive research has been done to try to uncover the riddle, the underlying molecular basis remains only partially understood, we hope that this proposed research will contribute to the greater general knowledge on the mechanism of AF development and break the vicious cycle of "AF Begets AF" by early autonomic intervention. Besides, if this hypothesis proven to be true, it would be beneficial to explain the link between obesity and AF [41], especially in patients with a higher volume of epicardial adipose tissue [42-44]. As research has revealed that obesity with an expansion of adipose tissue mass can result in the excessive secretion of tumor necrosis factor-alpha, which could induce the expression of NGF [45]. Therefore, this hypothesis is significative, it may help to elucidate the vicious cycle between autonomic remodeling and AF and to find new therapeutic targets for the treatment of AF.

Conflict of Interest Statement

None declared.

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