Angiotensin II-Superoxide Signaling and Arterial Baroreceptor Function in Type-1 Diabetes Mellitus

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Abstract

Diabetes is a major world health problem. Growing evidence from both clinical trials and animal experiments has clearly confirmed that arterial baroreflex dysfunction is a feature of type 1 diabetes, which links to prognosis and mortality of the type 1 diabetic patients. The arterial baroreflex normally regulates the blood pressure and heart rate through sensing changes of arterial vascular tension by the arterial baroreceptors in the aortic arch and carotid sinus. The aortic baroreceptor neuron located in the nodose ganglia is a primary afferent component of the arterial baroreflex. The functional changes of these neurons are involved in the arterial baroreflex dysfunction in the type 1 diabetes. Type 1 diabetes causes the overexpression and hyperactivation of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels and further reduces cell excitability of the aortic baroreceptor neurons. The alterations of the HCN channels are regulated by angiotensin II-NADPH oxidase-superoxide signaling in the aortic baroreceptor neurons. From the present review, we can understand the possible mechanisms responsible for the attenuated arterial baroreflex in the type 1 diabetes. These findings are beneficial for improving quality of life and prognosis in patients with the type 1 diabetes mellitus.

Keywords: Baroreflex; Baroreceptor; Ion channels; Angiotensin II; Superoxide; Diabetes

Introduction

The arterial baroreflex normally minimizes short-term oscillations in arterial blood pressure through regulating sympathetic and parasympathetic outflow [1,2]. Many studies have reported that type 1 diabetes causes the arterial baroreflex dysfunction in patients and animal models [3-16]. As a frequent complication of type 1 diabetes, the impairment of the arterial baroreflex contributes to high morbidity and mortality in type 1 diabetic patients [17-23].

In the arterial baroreflex arc, arterial baroreceptor neurons located in the nodose ganglia and petrosal ganglia are the main afferent component. These neurons sense the mechanical alteration of the arterial vascular walls through the baroreceptor terminals and increase the afferent neuronal excitation. This excited signal in the baroreceptor neurons is conveyed to the dorsal medial nucleus tractus solitary and evokes the cardio- and sympatho-inhibitory responses (such as decreasing peripheral vascular resistance and heart rate) [24-27]. Although it is possible that each component of the arterial baroreflex arc is involved in the impairment of the arterial baroreflex in type 1 diabetic condition, recent studies have indicated that the arterial baroreceptor neurons are involved in diabetes-related arterial baroreflex dysfunction [12,28,29]. The present review will mainly discuss the involvement of the arterial baroreceptor neurons in the arterial baroreflex dysfunction in type 1 diabetes, particularly the changes of arterial baroreceptor function and the possible cellular and molecular mechanisms responsible for these alterations.

Morphological and Functional Alterations of the Arterial Baroreceptor Neurons in Type-1 Diabetes Mellitus

The arterial baroreceptor neurons belong to the pseudo-unipolar neurons. This type of neurons has a soma located in the nodose or petrosal ganglia. An axon leaves the soma and further splits into two branches (30). One branch named as peripheral branch innervates the aortic arch and carotid sinus for sensing mechanical alteration of the

arterial vascular wall. Other branch named as central branch projects to the nucleus tractus solitary of the medulla for conducting the electrical signals of the baroreceptor neurons to the central nervous system [30]. So far it is still unclear how changes of the arterial vascular tension are converted into the electrical signal in the baroreceptor neurons. In general, there are mechanosensitive ion channels (such as epithelial sodium channels) in the baroreceptor nerve endings innervating to the aortic arch and carotid sinus [31]. The mechanosensitive ion channels sense the alterations of the arterial vascular tension and might convert the mechanical tension into the electrical signal. In the baroreceptor neurons, the electrical signal is conducted to the nucleus tractus solitary by neuronal excitation (action potential) that is controlled by voltage-gated ion channels (including sodium, calcium, and potassium channels). Therefore, it is possible that type 1 diabetes causes these electrophysiological changes (such as ion channel properties), which link to the blunted arterial baroreflex. However, determining the mechanotransduction in the arterial baroreceptor terminals imbedded in the vascular wall requires the development of advanced techniques, not yet available [32]. The neuron somata of the arterial baroreceptors are extensively used to investigate the potential mechanisms associated to the sensitivity of the arterial baroreceptors.

Type 1 diabetes-induced pathophysiological changes of the arterial baroreceptor neurons are generally divided into morphological changes and functional changes. An endless debate still remains about type 1 diabetes-induced morphological change due to different animal models and different time course of type 1 diabetes. Guo et al.

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have tested apoptosis in the vagus nodose ganglia using assessment of terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL)-positive and caspase-3-positive neurons [33]. They found that streptozotocin (STZ)-induced type 1 diabetes increased apoptotic-positive cells in the nodose ganglion neurons including the aortic baroreceptor neurons ($1.2 \pm 0.2\%$ of total neurons from diabetic rats vs. $0.2 \pm 0.08\%$ of total neurons from sham rats) [33]. Nevertheless, it is unclear whether low level of the apoptotic nodose neurons (about 1% of the total neuronal population [33]) can affect the sensitivity of the arterial baroreceptors in type 1 diabetes. On the other hand, some studies have confirmed that diabetes doesn't induce cell death of the nodose ganglion neurons [34], but it affects the expression of some neuroactive agents (such as increasing neuronal nitric oxide synthase-immunoreactive neurons, raising transcription factor c-Jun-immunoreactive neurons, and decreasing tyrosine hydroxylase-immunoreactive neurons) in the nodose ganglion neurons [35]. Our recent study indicates that STZ-induced type 1 diabetes doesn't alter the total cell number of the nodose ganglion neurons and the ratio of different subtype neurons [36]. Based on the above data, we consider that the impairment of the arterial baroreflex in the early stage of type 1 diabetes might be associated with the functional alterations in the baroreceptor neurons at the cellular and molecular levels but not the morphological changes. Of course, we do recognize that diabetes can induce morphological changes following the progression of type 1 diabetes.

As mentioned above, neuronal excitation is a key factor to affect the arterial baroreceptor function. Patch-clamp technique has been thought to be a feasible and powerful tool to investigate the electrophysiological properties of cell membranes in a number of the excitable cells including neuron cells. All major voltage-gated ion channels (including sodium channels, calcium channels, and potassium channels) are detected in the nodose neurons by the whole-cell patch clamp recording [37-41]. Although we understand that these voltage-gated ion channels are the key factors to affect the cell excitation of the baroreceptor neurons, there is no available information about the alterations of these channels in the arterial baroreceptor neurons from type 1 diabetes. Growing evidence from animal experiments has indicated that hyperpolarization-activated cyclic nucleotide-gated (HCN) channels might be a potential candidate to influence the cell excitation of the arterial baroreceptor neurons in normal and type 1 diabetic condition [36,42-44].

The HCN channels were first reported in 1976 and it was thought to be involved in the cell automaticity in the sinoatrial node of the heart [45,46]. The HCN channels have been divided into HCN1, HCN2, HCN3, and HCN4 isoforms by genomic classification [47-51]. Each channel isoform is also distinguished by distinct activation rates (HCN1 > HCN2 > HCN3 > HCN4) [52-56]. Additionally, some endogenous substances also modulate the HCN channel kinetics. For example, activation of the HCN channels is facilitated by cAMP and cGMP [52,56-59].

mRNA and protein of the HCN1-4 are expressed in the nodose ganglion neurons [36,47]. Additionally, the localization of HCN channel isoforms in the nodose ganglion neurons was measured by fluorescent immunohistochemistry [36,42]. Although the HCN3 and HCN4 proteins are expressed in all nodose neurons, HCN1 protein is only expressed in A-type (myelinated-fiber) nodose neurons and HCN2 protein is only expressed in C-type (non-myelinated-fiber) nodose neurons [36,42]. More importantly, the HCN channel protein is overexpressed in all nodose ganglion neurons from STZ-induced

type 1 diabetic rats, compared to sham rats [36,42]. Matching with the data for the expression of HCN channels, STZ-induced type 1 diabetes significantly increases the HCN currents in all aortic baroreceptor neurons [36,42,43].

The HCN channels are expressed in many tissues and have different physiological roles in these tissues. In the heart, the HCN channels trigger the initiation of the heart rate [45,60,61]. In various brain regions, the HCN channels are involved in the generation of sustained rhythmic oscillations [62-64]. In our recent studies, we found that HCN channel blockers (CsCl and ZD-7288) lower the HCN current density and raise the cell excitability in all aortic baroreceptor neurons (peripheral ganglion neurons) [42,43]. From our studies, we claim that the role of the HCN channels in the peripheral baroreceptors is distinct from their roles in the heart and brain. One possible reason is that the peripheral baroreceptor neurons are non-spontaneously exciting cells and their excitation needs the exciting signal from their afferent endings [42,65]. Additionally, STZ-induced type 1 diabetes increases the HCN currents and decreases the cell excitability in all isolated aortic baroreceptor neurons, compared to those in sham condition [36,42,43]. These data further confirm that activation of the HCN channels decreases the cell excitability in the baroreceptor neurons.

Modulatory Effect of Angiotensin II-Superoxide Signaling on HCN Channels in the Arterial Baroreceptor Neurons from Type 1 Diabetes Mellitus

Angiotensin (Ang) II has been recognized as a physiologically active peptide in multi tissues including nodose ganglia [66,67]. This endogenous hormone has some physiological effects such as the maintenance of blood pressure and fluid homeostasis [68]. Normally these physiological actions of Ang II are mediated by Ang II receptors located on the cells of local tissues [69]. It is known that circulating and local tissue Ang II concentrations are elevated in diabetic patients and animal models with diabetes [70-72]. Using some techniques including autoradiography, nodose ganglionectomy, and vagal ligation, Allen, et al. found that Ang II receptor binding sites existed in somata of the nodose neurons and transported to terminals of the nodose neurons [66,73]. Electrophysiological study revealed that exogenous Ang II has the direct neuronal effect on the nodose neurons through Ang II type 1 (AT_1) receptors [74]. The data from radioimmunoassay, single-cell RT-PCR, and western blot demonstrated that endogenous Ang II level and expression of AT_1 receptor mRNA and protein are increased in the nodose neurons from STZ-induced type 1 diabetic rats [43]. Additionally, exogenous Ang II mimics diabetes to enhance the HCN currents and to lower the neuronal excitability in the isolated aortic baroreceptor neurons from sham rats [44]. More importantly, diabetes- or exogenous Ang II-induced alterations of the HCN currents and neuronal excitability in the isolated aortic baroreceptor neurons are fully normalized by AT_1 receptor antagonists (losartan and L158,809) [43,44]. These results demonstrate that the elevation of endogenous Ang II can attenuate excitation of the aortic baroreceptor neurons through activating HCN channels in type 1 diabetes.

Above electrophysiological results were obtained in the fresh dissociated single neurons. Now question is how endogenous Ang II existed in the cytosol modulates the HCN channel kinetics in a dissociated single neuron. In general, Ang II binds with AT_1 receptors located in the cell membrane, and then induces cellular responses through activating intracellular signaling cascades. However, intracellular Ang II level was elevated in renal cortical endosomes during exogenous Ang II-induced hypertension [75]. In cardiac

myocytes isolated from hamsters, intracellular administration of Ang II influences the electrophysiological properties of the voltage-gated calcium channels and the cell junctional conductance [76,77]. Additionally, the effect of intracellular Ang II on ion channels and cell junctional conductance is abolished by intracellular treatment of losartan (a selective AT₁ receptor antagonist) but not extracellular losartan [76,77]. We also observed that intracellular administration of losartan (added to the recording pipette solution) decreased the HCN current density and increased the cell excitability in the aortic baroreceptor neurons from diabetic rats [43]. Therefore, it is possible that diabetes activates an intracellular angiotensin II production system in the nodose ganglia. Of course, we couldn't neglect the effect of circulating Ang II on the neurons when we analyze and explain the effect of Ang II in the whole animal experiments or clinical phenomena.

What mechanism(s) account for Ang II-induced activation of HCN channels? Ang II binds with AT₁ receptors to cause the superoxide production mainly through activation of NADPH oxidase [78]. NADPH oxidase is a multicomponent enzyme that produces the superoxide by the one-electron reduction of oxygen. This enzyme has two cell membrane-associated subunits (gp91^{phox} and p22^{phox}), three cytosolic subunits (p40^{phox}, p47^{phox}, and p67^{phox}), and the small G-proteins (Rac and Rap1a) [79,80]. As a main source of the intracellular superoxide, NADPH oxidase has been found in many tissues [81-86]. Protein of the NADPH oxidase subunits is also expressed in the nodose ganglia [43]. Diabetes increases the protein expression of all NADPH oxidase subunits in the nodose ganglia [43]. NADPH oxidase inhibitors (apocynin and phenylarsine oxide) or superoxide scavengers (tempol and polyethylene glycol-superoxide dismutase) significantly inhibit diabetes- or exogenous Ang II-triggered overproduction of the superoxide, hyperactivation of the HCN channels, and suppression of the cell excitation in the aortic baroreceptor neurons [43,44]. The data from these studies clearly suggest that Ang II-NADPH-derived superoxide signaling regulates the neuronal excitability through influencing activation of the HCN channel in the aortic baroreceptors from type 1 diabetic rats.

Normally a low level of the superoxide in the healthy cells is produced by leakage or short circuiting of electrons [87]. This tiny amount of the superoxide is able to kill microorganisms and to clean some cellular wastes [87,88]. Our recent study found that superoxide scavenger (tempol and polyethylene glycol-superoxide dismutase) did not affect activation of the HCN channels and cell excitability although they inhibited basal superoxide production in the aortic baroreceptor neurons from sham rats [43,44]. However, after acute administration of these chemicals in the aortic baroreceptor neurons from type 1 diabetic rats, the HCN currents and the cell excitability were normalized to the levels seen in the sham neurons [43]. One reasonable explanation is that only a higher level of the superoxide production can modulate the electrophysiological properties of the HCN channels.

An antioxidant, α-lipoic acid prevents diabetes-induced arterial baroreceptor dysfunction [89]. Are all endogenous oxidants involved in the attenuation of the arterial baroreceptor function in type 1 diabetes? Several reactive oxygen species (ROS) including superoxide, hydrogen peroxide, and hydroxyl radical are biologically derived from oxygen [90]. These chemically reactive molecules are highly unstable and short-lived [90]. In general, superoxide dismutase rapidly catalyzes two superoxide anions into a molecule of hydrogen peroxide, which in turn is quickly reduced to hydroxyl radical. The electrophysiological properties of ion channels are modulated by these reactive molecules [91-93]. In the aortic baroreceptor neurons, superoxide scavengers

(tempol and polyethylene glycol-superoxide dismutase) have an ability to normalize diabetes-enhanced HCN currents to the level seen in sham neurons [43]. These data indicate that overproduction of superoxide but not hydrogen peroxide and hydroxyl radical is involved in the modulation of the HCN channels in the aortic baroreceptor neurons from STZ-induced type 1 diabetic rats.

The electrophysiological properties of the ion channels can be modulated by most of the endogenous substances. The modulation of the ion channels is divided into acutely influencing the kinetics of the ion channels (such as changing activation and inactivation curves) and chronically altering the expression of the ion channels (including mRNA and protein). In the studies described above, the acute effect of Ang II-NADPH oxidase-superoxide signaling on the kinetics of the HCN channels was measured in the aortic baroreceptor neurons from sham and STZ-induced diabetic rats [43,44]. Our recent study found that this signaling pathway also affects the expression of the HCN channel protein in rat nodose neurons [94]. These findings, taken together, demonstrate that development of type 1 diabetes activates Ang II-NADPH oxidase-superoxide signaling cascade and resultant overexpression and hyperactivation of the HCN channels in the aortic baroreceptor neurons.

Involvement of Ang II-Superoxide-HCN Channel Signaling in Impairment of the Arterial Baroreflex in Type 1 Diabetes Mellitus

One recent study has shown that the impaired baroreflex sensitivity correlates with the changes in the nucleus tractus solitary neural firing rates, which is affected by both afferent nervous behavior and nucleus tractus solitary cells themselves in the STZ-induced type 1 diabetic rats [95]. The collision experiments in the rabbit nodose ganglia have found that somata and central axons of the nodose neurons modulate the visceral afferent neuronal signals to the central nervous system [96]. One review paper has concluded that nodose ganglion neurons can be able to integrate the various received signals for the modulation of vago-vagal reflex [97].

A method for evaluation of the arterial baroreflex is to examine the changes of blood pressure and heart rate induced by electrical stimulation of baroreceptor-containing nerve (aortic depressor nerve),

which is widely accepted by researchers [98-100]. The advantages for this method include: 1) the aortic depressor nerves of rabbits, rats, and mice contain only baroreceptor afferent fibers and no functional chemoreceptor afferent fibers for the transmission of input signals [101-104]; 2) direct electrical stimulation of the aortic depressor nerve is to bypass the mechano-viscoelastic coupling between changes in the aortic arterial vascular walls and afferent nerve endings; 3) easily changing the frequency and intensity of stimulus can differentiate the reflex responses to activating A- and C- afferent fibers. Our studies have shown that type 1 diabetes significantly attenuated the arterial baroreflex sensitivity measured by the reflex responses of blood pressure and heart rate to the electrical stimulation of the aortic baroreceptor nerves [12,105]. Additionally, local microinjection of exogenous Ang II into the nodose ganglion could mimic the diabetes to decrease the arterial baroreflex sensitivity [12]. Furthermore, our studies also found that microinjection of AT₁ receptor antagonist, NADPH oxidase inhibitor, superoxide scavenger, or HCN channel blocker into the nodose ganglion significantly improved diabetes- or exogenous Ang II-induced impairment of the arterial baroreflex sensitivity [12,105]. These data provide the direct evidence that Ang II-superoxide-HCN channel signaling contributes to impairment of the arterial baroreflex sensitivity in STZ-induced diabetic rats.

In this review, we have updated the new information about the functional alterations of the arterial baroreceptors in type 1 diabetes. Ang II/AT₁ receptor-NADPH oxidase-superoxide signaling modulates the expression and activation of the HCN channels in the aortic baroreceptor neurons and subsequently causes the impairment of the arterial baroreflex in type 1 diabetes (Figure 1). These new findings provide potential therapies for improving the prognosis of patients with type 1 diabetes.

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