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Role of Angiotensin \(\text{II}\) in Nucleus Tractus Solitarius

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Key wards: Nucleus tractus solitarius/ calcium channel currents/ Angiotensin \(\text{II}\)

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34 pages

1 figure and figure legend

Running title: Ca\(^{2+}\) channel modulation by angiotensin \(\text{II}\) in NTS
Abstract: There are local renin-angiotensin systems (RAS) in region such as the kidney and heart, and that dysfunction of these systems may lead to changes in the regulation of blood pressure. Recently, it is likely that all components of the RAS are present in the brain, and local production of angiotensin peptides has been shown in several brain areas. Stimulation of brain RAS leads to increases in blood pressure, attenuation of the baroreceptor heart-rate reflex, stimulation of drinking, and release of various hormones including vasopressin.

Nucleus tractus solitarius (NTS), located within the dorso-medial medulla, is the site of termination for primary afferent fibers originating from a wide variety of peripheral organs and tissues and is essential in integration of autonomic nervous system functions.

Several demonstrations suggest that the NTS contains all of the components of the RAS including angiotensinogen, angiotensin-converting enzyme (ACE), Angiotensin II (Ang II) and Ang II receptors. This review reports the role of Ang II in NTS. (161 words)
Introduction

Nucleus tractus solitarius (NTS)

Nucleus tractus solitarius (NTS) plays a major role in the regulation of cardiovascular, respiratory, gustatory, hepatic and swallowing functions \(^1-^3\). This nucleus receives primary afferent input from a wide variety of peripheral organs and tissues and is essential in integration of autonomic nervous system functions. In accordance with the numerous integrative functions mediated by the NTS, over 30 neurotransmitter/neuromodulators have been found in both cell bodies and terminals within the NTS \(^2\).

Located in the dorsomedial aspect of the medulla oblongata, the NTS plays a pivotal role in reflex control of autonomic homeostasis \(^4,^5\). The NTS appears not to be a simple relay nucleus, rather it performs complex integration of information from multiple synaptic inputs of both peripheral and central origins \(^6-^8\). Understanding the NTS and its role provides an opportunity to reveal mechanisms of integration and modulation in the central nervous system (CNS).
**Cardiovascular regulatory mechanisms via NTS**

The baroreflex is the main sensory mechanism involved in the central neuronal control of the circulation and NTS is the site of the first synapse of baroreceptor afferents in the CNS \(^9\). NTS is an important synaptic station of the cardiopulmonary vagal afferents in the central nervous system and plays a key role in the modulation of the autonomic efferent activity to the cardiovascular system \(^10\)-\(^12\).

The baroreceptors are stimulated by distention of the structures in which they are located, and so they discharge at an increased rate when the pressure in these structures rises. Their afferent fibers pass via the glossopharyngeal nerves (\(\uparrow\)) and vagus nerves (\(\downarrow\)) to the medulla. Most of them unite in the NTS. There are also excitatory projections, probably polyneuronal, from the NTS to the vagal motor neurons in the dorsal motor nucleus and the nucleus ambiguous. Thus increased baroreceptor discharge inhibits the tonic discharge of the vasoconstrictor nerves and excites the vagal innervation of the heart, producing vasodilation, venodilation, a drop
in blood pressure, bradycardia and a decrease in cardiac output.

Cardiac primary afferents running in the sympathetic nerve, especially finely myelinated Aδ- and unmyelinated C-fiber afferents, are considered to be the essential pathways for transmission of cardiac nociception to the central nervous system13-15). Axonal tracing studies have shown that cardiac sympathetic afferents project to the dorsal horn of the upper thoracic spinal cord through the stellate ganglia and the sympathetic chain13,16). Stimulation of cardiac afferents excites neurons in the spinal ascending pathways, such as those located in the spinothalamic tract17). The vasomotor neurons in the rostral ventrolateral medulla (RVLM) provide critical excitatory output to the preganglionic sympathetic neurons18,19). Stimulation of NTS neurons typically modulates sympathetic outflow through excitation of neurons in the caudal ventrolateral medulla, which, in turn, inhibits vasomotor neurons in the RVLM10,20,21).

**Taste pathways via NTS**

Sapid tastants depolarize taste receptor cells in the tongue22). The sensory
nerve fibers from the taste receptor cells on the anterior two-thirds of the
tongue travel in the chorda tympani of the facial nerve (‡), and those from
the posterior one-third of the tongue reach the brain stem via the
glossopharyngeal nerve (‡). The fibers from areas other than the tongue
reach the brain stem via the vagus nerve (‡). On each side, taste fibers in
these three nerves unite in the gustatory portion of the NTS 23,24, where the
initial processing of taste quality and intensity occurs. From there, axons of
second-order neurons ascend in the ipsilateral medial lemniscus and pass
directly to the ventral posteromedial (VPM) nucleus of the thalamus. From
the thalamus, the axons of the third-order neurons pass in the thalamic
radiation to the face area of the somatosensory cortex in the ipsilateral
postcentral gyrus. They also pass to the anterior part of the insula.

**NTS neurons in swallowing**

Swallowing is a reflex response that is triggered by afferent impulses in
the trigeminal nerve (‡), glossopharyngeal nerve (‡) and vagus nerve (‡).
These impulses are integrated in the NTS that have a centrally patterned
activity 25,26). The efferent fibers pass to the pharyngeal musculature and the tongue via the trigeminal nerve (‡X) and hypoglossal nerve (‡U). Anatomical studies have shown projections to the NTS from trigeminal (‡X) afferents 23,27,28). Within the NTS, there exist neurons that fire during either the oropharyngeal or the esophageal phase of swallowing. These neurons exhibit a typical sequential firing pattern that parallels the sequential motor pattern typical of deglutition. When rhythmic oropharyngeal phases of swallowing are elicited, NTS neurons involved in the oropharyngeal sequence produce rhythmic bursting discharges that are closely linked to the motor pattern. Because these neurons are still active during fictive swallowing elicited, their bursting discharge cannot be due to peripheral afferent inputs generated by the muscular contraction and actually correspond to a central swallowing activity 29). These studies demonstrate that NTS medullary neurons form part of the neuronal network that generates swallowing.

Most of the data on the intrinsic properties of neurons liable to be involved in swallowing have been obtained in studies on brain stem slices. The data indicate that neurons located in dorsal and ventral medullary regions and in
cranial motor nuclei that are involved in swallowing, such as trigeminal (‡X), facial (‡Z), vagus (‡\] and hypoglossal (‡\]‡U) motor nuclei and the nucleus ambiguous, possess various ionic conductances that may be involved in patterning the swallowing motor event \(^{30-39}\).

**Ionic channels in NTS**

Multiple ionic channels are known to be involved in the regulation of neuronal excitability \(^{40,41}\). Several studies have reported that NTS possesses Ca\(^{2+}\)-dependent K\(^+\) channels (K\(_{ca}\)) \(^{33,42-44}\), voltage-dependent Ca\(^{2+}\) channels (VDCCs) \(^{45,46}\), Cl\(^-\) channel \(^{47}\), K\(^+\) channel \(^{49}\), ATP-activated K\(^+\) channel \(^{49}\) and non-selective cation channel \(^{50}\).

**Voltage dependent Ca\(^{2+}\) channels (VDCCs)**

Historically, VDCCs were first identified in crustacean muscle by Fatt and Katz \(^{51}\), and their properties were examined by several groups \(^{52,53}\). VDCCs have subsequently been found in all types of excitable cell in vertebrates and
invertebrates, and even plants.

Many important cellular functions are controlled by the concentration of intracellular free Ca\(^{2+}\) ([Ca\(^{2+}\)])\(_i\). The concentration of Ca\(^{2+}\) ion in the cell therefore is closely regulated by a number of systems which maintain free Ca\(^{2+}\) levels within narrow limits. One major mechanism for elevating Ca\(^{2+}\) concentrations resides under the control of voltage sensitive, Ca\(^{2+}\) permeable pores or VDCCs in the cellular membrane. These VDCCs function in the resting state, to maintain the extracellular Ca\(^{2+}\) concentration at about 10,000 times higher than that found inside the cell. The invasion of an electrical or other type of depolarizing impulse into the cell results in the opening of VDCCs for brief periods. This sudden transient influx of Ca\(^{2+}\) triggers a number of intracellular biochemical events.

**Angiotensin**

Angiotensin (Ang) has various physiological effects mediated by the brain, including stimulation of increased blood pressure, water and sodium intake, vasopressin secretion, and modulation of baroreflex function.\(^{54,55}\)
Ang also acts as a neurotransmitter in the central nervous system mediated by G-protein coupled receptors (GPCRs) \(^{56}\). There is a high density of AT\(_1\) receptors in the NTS \(^{57,58}\), which are located on both neuronal cell bodies and vagal afferent fibers within the nucleus \(^{59-63}\).

**Signal pathways of Angiotensin II receptors**

The effects of Ang on cell signaling and ionic homeostasis have been documented extensively in many cell types \(^{64}\). In muscle cells, Ang stimulates phospholipase C (PLC), the production of inositol 1,4,5 trisphosphate (IP\(_3\)) and diacylglycerol (DAG), and the mobilization of [Ca\(^{2+}\)]\(_i\) \(^{65}\). In neurons, one of the better characterized pathways involves activation of phosphoinositide and increased Ca\(^{2+}\) release from intracellular stores \(^{66}\). The increase in Ca\(^{2+}\) then leads to activation of a wide variety of pathways that mediate the short- and long-term effects of Ang \(^{64}\).

**Angiotensin II modulate VDCCs in NTS-Physiological relevance**
In NTS, the author demonstrated that Ang II facilitated L-type VDCCs via G protein-involved Src tyrosine kinase and p38 mitogen-activated protein kinase (MAPK) kinase mediated by AT₁ receptors. Additionally, Wang et al. have demonstrated that NADPH oxidase-derived reactive oxygen species (ROS) are involved in Ang II-induced facilitation of L-type VDCCs in NTS (Fig. 1).

Ang II-induced facilitation of L-type VDCCs might be the regulation of Ca²⁺-dependent enzymes, in particular, transcription factors. L-type VDCCs have been linked to not only somal action potentials, but also the activation of Src, the modulation of receptor-linked Protein Tyrosine Kinase, and gene expression, including synthesis of ion channels. During differentiation, L-type VDCCs-mediated Ca²⁺ influx is essential for neurite outgrowth, synapse formation, survival, and the shift to the mature action potential profile. Developmentally, Ca²⁺ influx would be expected to trigger influx-dependent cellular differentiation and, because early action potential activity has been linked to establishing neuronal circuits, potentially could contribute to pattern information. This study strongly imply that Ang II-induced facilitation of L-type VDCCs would lead to a
substantial increase in Ca\(^{2+}\) influx. Whether occurring as a result of spontaneous activity or mature action potential firing, such increases in [Ca\(^{2+}\)], may dramatically alter neuronal function.

It has become evident that tyrosine phosphorylation is not only crucial for regulation of growth-related responses such as gene transcription and cell division, but that it is also important for rapid cellular responses as cell adhesion \(^{75}\) and migration \(^{76}\). Ang\(^{\perp}\)-induced MAPK activation stimulates activation of various transcription factors, including Fos and Jun, have been demonstrated \(^{77,78}\). It is also accepted that non-RTK activated by Ang\(^{\perp}\) has pivotal role in neuronal long term events \(^{79}\). Ang\(^{\perp}\) activation of Src tyrosine kinase can lead to MAPK activation and the induction of the transcription factors, fos, jun and myc, resulting in an increase in gene transcription \(^{80}\). It is clear that the physiological actions of Ang\(^{\perp}\) are associated with increased expression of the proto-oncogenes c-fos and c-jun (and their protein products c-Fos and c-Jun) in the brain regions \(^{81-84}\). The transcriptional activity of c-Fos and c-Jun is augmented by phosphorylation \(^{85-87}\). Several kinases are responsible for phosphorylating and regulating c-Fos and c-Jun. For example, c-Fos protein can be phosphorylated by Erk 1
and Erk2 \(^{88}\). In contrast, c-Jun is phosphorylated by JNKs \(^{89}\). Ang\(-\)activated ERK1/2 is responsible for the induction of early growth response genes, whose family includes the proto-oncogenes c-fos, c-jun and c-myc\(^ {90,91}\). Protein products of these genes are involved in signaling cascades resulting in the growth response.
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Figure Legend

Schematic diagram of the signaling pathways responsible for Ang-Ⅱ-mediated facilitation of VDCCs in NTS. Abbreviation for intracellular moleculars: α, β and γ, G-protein subunits; PLD, phospholipase D; PIP2, phosphatidylinositol-4,5-bisphosphate; DAG, diacylglycerol; PKC, protein kinase C; MAPK, mitogen-activated protein kinase; VDCCs, voltage-dependent Ca2+ channels.
L-type VDCCs

AT₁ receptor

gp91

(+)

p47 MAPK

p43 MAPK

p38 MAPK

Src kinase

PLD

DAG

PIP₂

PKC