LINKAGES OF Ca V1.2, GLUTAMATE, AND ODONTOBLAST
IN THE MECHANISM OF TOOTH PAIN

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ABSTRACT

Background: Odontoblast is often associated with its role as sensory cell in tooth pain. Odontoblasts have ion channels that contribute to the sensitivity and release of neurotransmitters in odontoblast stimuli that are activated in pulp sensory nerve fibers. Review: Ca V1.2 has unexpected plasticity. In dental injury, the appearance of Ca V1.2 canal in odontoblast is known to change, depend on the duration of injury. The dentinal pulp tissue has the ability to release glutamate, which acts as an intercellular mediator to create neuronal signaling communication between inter-odontoblast and odontoblast-trigeminal ganglion nerve (TG). Discussion: Odontoblasts as a mechanosensitive sensory cell are indicated by the role of the TRP transduction receptor and the release of ATP. Though other canals and active compounds in odontoblast are involved, an important role in delivering the sensation of pain also needs to be known. Odontoblast will communicate with paracrine pulp nerves using ATP and glutamate. Ca2+ enters the odontoblast through activated TRP channels and other ion channels, such as L-type VGCC channels (Ca V 1.2). Followed by the release of glutamate from odontoblast through the glutamate-permeable canal, it can trigger the pulp nerve via glutamate metabotropic receptors (mGluRs). Conclusion: There is involvement of Ca V1.2 and glutamate canals in odontoblast in the delivery mechanism of pain.

Keywords: Ca V1.2, Glutamate, Odontoblasts, Tooth Pain

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INTRODUCTION

Pain is a protective mechanism to raise awareness that tissue damage is occurring or will occur. The mechanism of pain onset is based on multiple processes namely nociception, peripheral sensitization, phenotype changes, central sensitization, ectopic excitability, structural reorganization and decreased inhibition.1 There are four separate processes between tissue injury stimulus and subjective experience of pain, namely transduction, transmission, modulation, and perception.2

Tooth pain is the most common symptom associated with dental problems and significantly determines oral health related to quality of life.3 It is usually caused by several stimuli including temperature, tactile, osmotic and chemical on the surface of exposed dentin.4 Those external stimuli are received by the enamel-dentin junction that does not contain nerve endings.5 The nerve endings enter the pulp end of the dental tubule. The pulp nerve branching extends only to the third layer of dentine, so that the next process is the hydrodynamic theory by generating stimulation from dentin surface into the dental tubule in form of fluid flow.

Odontoblasts are dental pulp cells that are responsible for dentin formation. It will migrate beneath the oral epithelium and into cells that play role in the regulation and organization of the mineralized matrix of dentin synthesis.6 Odontoblasts are often associated with their role as sensory cells in the incidence of tooth pain. This is related to several studies that odontoblasts are found in many ion channels, which contribute to the sensitivity and release of neurotransmitters in odontoblast stimuli. Those are activated in sensory pulpal nerve fibers.7

Ca2+ is one of some ions that plays a role in signal transduction from external stimuli in the mechanism of tooth pain. The Ca V1.2 canal is a type of Voltage-gated Calcium Channels (VGCC) which is expressed in the sensory system of the tooth.8 The activity of glutamate, the activated neurotransmitter, and calcium ion channels in odontoblast are interrelated to the tooth pain mechanism. The purpose of this article is
to inform that odontoblasts play an important role by study the activity of Ca V1.2 and glutamate which is involved in excitatory delivery in the tooth pain mechanism.

**LITERATURE REVIEW**

1. Ca V1.2 mediates Intracellular Ca$^{2+}$ influx in Odontoblasts

Ca$^{2+}$ is a second messenger that is widely used in various cell functions. The concentration of Ca$^{2+}$ in the cytosol is very small (10-20 nM), while the extracellular compartment is 1-2 mM. Ca$^{2+}$ canal opening causes elevation in intracellular Ca$^{2+}$ levels up to 100µM, which trigger various cellular processes, such as muscle contraction events, secretary cell exocytosis and neurotransmitter release in nerve cells. Ca$^{2+}$ has a role to activate the fusion of vesicles containing neurotransmitters with the plasma membrane, so that the neurotransmitters in nerve cells can be released from vesicles to cell compartment.

One of the Ca$^{2+}$ ion channels, VGCC, has an important part in the mechanism of tooth pain. VGCC is activated due to the influx of sodium channels. This activation will direct calcium influences according to the high concentration gradient between the two plasma membranes. The VGCC is classified into high VGCC, including L type (CaV1.1, CaV1.2, CaV1.3, CaV1.4), N type and R type, and low VGCC, including T-Type (CaV3.1, CaV3.2, CaV3.3).

In odontoblast, Ca$^{2+}$ L-type ion channel (Ca V1.2) is activated by a large depolarization, and open up long enough before being inactive (500 m seconds or more). Ca V 1.2 is known to have unexpected plasticity. In the event of a dental injury, the appearance of the Ca V1.2 canal in odontoblast varies based on the duration of injury. It can also be associated with pain that appears on the tooth, due to the presence of inflammatory process. Ca V1.2 has an important part in odontoblast activity, physiologically and pathologically.

Previous studies have found that there are several ion channels and receptors which are localized to cellular primary afferent neurons (DPAs) and odontoblasts. Those involve in the transduction of tooth pain. Intracellular signal transduction between odontoblast and TG occurs along with the presence of odontoblast mechanical stimulation. The mechanical stimulation increases intracellular Ca$^{2+}$ concentrations by intermediate activation of the mechanosensitive-transient receptor potential (TRP) channels, such as TRPV1, TRPV2, TRPV4, and TRPA1.

Increased intracellular Ca$^{2+}$ also happens not only in odontoblasts, but also in adjacent cells. Ca$^{2+}$ influx is followed by activation of TRPV1. TRPV1 is mediated by the functional entry of Ca$^{2+}$ pairs with CB receptor activation via cAMP signalling. cAMP mediated crosstalk CB1-TRPV1 and TRPV1-NCX coupling play an important task in the delivery of cellular functions followed by transduction of external and thermal stimuli in odontoblasts. TRPV1 is one of the specific nociceptive receptors present in terminal afferent neurons. The mechanism of excitatory delivery involves terminal afferent sensitization followed by changes in Ca$^{2+}$ ions and activation of coupled G-protein couplers (GPCRs) that result in changes in adenyly cyclase (AC)/cAMP/PKA, phospholipase C (PLC) / inositol triphosphate (IP3)/Ca$^{2+}$ or receptor activity and affect the activity of AC/cAMP/PKA, PLC/IP3/Ca$^{2+}$ or PLC / DAG / PKC.

The intracellular Ca$^{2+}$ accessibility may causes stress on the endoplasmic reticulum and mitochondrial DNA, then affects ROS and ATP reduction. Although there is no synaptic structures were found between odontoblasts and afferent nerves, ATP and glutamate were proposed as signal mediators between odontoblasts and neural afferents. TRP receptors in odontoblasts receive hydrodynamic pressure and ATP release in pulp neurons to produce tooth pain. Ca V1.2 temporarily mediates Ca$^{2+}$ in conditions of persistent tooth pain.

2. Glutamate as Signal Mediator in Odontoblasts

There are three categories of pain receptors or nociceptors. Mechanical nociceptors respond to mechanical damage, temperature nociceptors respond to extreme temperatures, and polymodal nociceptors that respond equally to all types of damaging stimuli, including chemical irritant which is released by injured tissue. In response to potential actions caused by stimuli, afferent pain fibers secrete neurotransmitters that affect subsequent neurons. The most known neurotransmitters are the substandard P and glutamate. The P substance activates ascending pathways which transmit nociceptive signals to higher levels for further processing. Ascending pain pathways have different goals in the cortex, thalamus, and reticular formation.

Glutamate is the main excitatory neurotransmitter released from primary afferent terminal pain nerves. Glutamate is derived from glutamine precursors produced by glia cells. Glutamate works on two different plasma membrane receptors in dorsal end neurons, with two different effects. First, binding of glutamate with the AMPA receptor causes changes in permeability which ultimately leads to the formation of action potentials in dorsal end cells. This action potential transmits pain messages to higher centers. Second, binding of glutamate with NMDA receptors provokes the entry of Ca$^{2+}$ into the dorsal end. This pathway is not involved in pain message transmission. Ca$^{2+}$ generates the second messenger system which makes post synaptic neurons more sensitive than usual. This hyper accessibility has a role in increasing the sensitivity of the injured area to impulse pain stimulation. Glutamate increases the sensitivity of the post synaptic neuron.
region, so that there is an increase in the responsiveness of the peripheral receptors to pain relief, and the receptor reacts stronger to the next stimulus.23

Odontoblasts also express glutamate receptors (GluRs) in group I, II, and III mGluRs. N-methyl-d-aspartic acid (NMDA) receptor is not involved in interodontoblast communication. This means that the dentinal pulp tissue has the ability to release glutamate, which acts as an intercellular mediator to create neuronal signaling communication between interodontoblast and odontoblast-TG.22

The release of glutamate neurotransmitters in terminal axons is produced by the entry of Ca\(^{2+}\) into cells and nociceptive signals which are then sent and carried through different neurons in action potential processes.1 Glutamate concentrations in synaptic gaps are maintained by Na\(^{+}\)-dependent, high affinity transporters namely Excitatory Amino Acid Transporters (EAATs) which is located in neurons and glia. A precise proportion of the amount of glutamate transporters is needed to determine the excitatory signal and prevent excitotoxic neural damage. In addition to act as a neurotransmitter, glutamate can be a neurotoxin that includes the role of transporters.24

Odontoblast is enriched with glutamate, which acts as a neuroactive substance. Released in adjacent pulp neurons in high level, it shows immunoreactivity to TRPV1, TRPA1, and TREK-1.25 The adjacent sensory pulp expresses mGluR5 in odontoblasts. Glutamate level in odontoblast and mGluR5 receptor expression around axon may be gradually triggered as tooth pain arises.25

**DISCUSSION**

The nerves that entering the dental pulp consist of sensory fibers and sympathetics postganglion which originate from trigeminal neurons and sympathetic superior ganglia. These neurons will supply blood vessels, pulp cells and odontoblasts through fibers called subodontoblastic plexus. The odontoblast innervation pattern is directly regulated by local nerves and nerve repulsive molecules such as growth factors, glial cells, sema7A proteins or reelin, which released by the pulp and odontoblast cells themselves. These molecules will move from the nerve terminal to the appropriate location.7

The dentin frame and tubules in odontoblast are innervated by myelinated A and unmyelinated C sensory nerve fibers.3, 26 The central ends of the Ad- and C fibers release some excitatory neurotransmitters and stimulate nociceptive neurons. This will initiate the central nerve to process nociceptive information on the cortex and limbic circuit to produce sensory perception which will be transmitted through several nuclei on the brain stem and thalamus.26, 27

During this time, the role of odontoblasts as a mechanosensitive sensory cell is indicated because of transduction part from the TRP receptor and ATP release. Complex communication in the dental system cannot be focused on only one channel or one molecular component. In fact, there are other channels and other active compounds that also have an act in the mechanism of delivering tooth pain that need to be further investigated. Several findings indicate the involvement of CaV1.2 and glutamate in odontoblasts in the mechanism of sensors delivery.

Stimulus that appears in the exposed dentin causes movement in the dentinal tubule fluid, which activates the TRP channel's mechanosensitive receptors on the odontoblast and pulp nerve. Intense thermal stimulation will induce mechanical stress on the tooth structure that triggers TRP activity. Odontoblast will communicate with paracrine pulp nerves using ATP and glutamate. Ca\(^{2+}\) enters the odontoblast through the activated TRP and other ion channel, like VGCC.10 It means that the TRP is not the only access to Ca\(^{2+}\) influx into the odontoblast, but there are other ions such as ligand-gated and voltage-gated. The L-type VGCC that expressed is Ca V1.2, because its reaction may happen long enough in a state of persistent pain. This channel existence may change along with an exposure to stimulus, so this channel is difficult to detect.

Several mechanosensitive ion channels are expressed by the sensory system of the tooth and involve the theory of hydrodynamics via TRPM7, which found in many odontoblasts. At the moment, PIEZO receptors are also found to be opening candidates. Several ligand-gated and voltage-gated ion channels are expressed in primary dental afferent neurons that are related to their role in the mechanism of tooth pain.10 Ca\(^{2+}\) ion channels that involved in the delivery of pain sensations is VGCC. VGCC Ca\(^{2+}\), Na, and K expressions, store-operated calcium channels and Na / Ca\(^{2+}\) exchangers indicate excitability of odontoblasts. VGCC is a transducer that is characterized by the presence of electrical activity that allows Ca\(^{2+}\) to enter the cell in response to the action potential or sub-threshold depolarization stimulation.28 The subtype of VGCC that is also expressed in the dental system is the L-type Ca\(^{2+}\) channel (CaV1.2). In addition to influencing Ca\(^{2+}\), CaV1.2 is also known to play a role in the differentiation and mineralization of dental apical papilla stem cells (rSCAPs) in mice.29

After the influx of Ca\(^{2+}\), ATP is then released from odontoblasts through the pannexin-1 channel (PANX-1) and may activate P2X receptors on the pulp nerve, which are then involved in detecting hydrodynamic pressure in the dentinal tubule.30 ATP will be converted by NTPDase into ADP, which can activate P2Y receptors on the pulp nerve. Then, glutamate released from odontoblast through the
glutamate-permeable canal may trigger the pulp nerve via the glutamate metabotropic receptor (mGluRs).  

Glutamate neurotransmitters involvement in intercellular odontoblast communication is to modulate the transduction of tooth pain signals. This is proved by the expression of group I metabotropic glutamate receptors that appear together with increased Ca\(^{2+}\) in odontoblast.  

In Cho et al study, extracellular glutamate concentration significantly increased in odontoblast line cell cultures treated with calcium permeable ionophore. This further strengthens suspicion that glutamate is released from odontoblast, mediated by the presence of intracellular Ca\(^{2+}\) influx. The presence of glutamate in the nociceptive receptor pathway is also supported by previous research that administration of eugenol’s active compound can overcome pain by inhibiting the nociceptive receptor pathway induced by glutamate.  

Odontoblasts that receive mechanical stimulation have the ability to release glutamate into the extracellular cleft via the glutamate-permeable anion channel. Glutamate release activates mGluRs in paracrine/autocrine odontoblasts, forming interodontoblast communication. Glutamate and mGluRs also mediate neurotransmission between odontoblasts and neurons in dental pulp to modulate sensory signal transmission. Glutamate in the synaptic cleft is adjusted by the presence of glutamate transporters in the presynaps, postsynaps, and astroglial cell membranes. Astroglial cells release glutamine, which is then taken up by terminal presynaps. There will be constant stimulation of the dental pulp nerve that experiences hypersensitivity associated with the synthesis of astroglial glutamine. This system is called an alternating glutamate-glutamine reaction, which can maintain homeostasis and delivery of pain on the side of the synapse. This further proves that in addition to ATP, glutamate has an important role in odontoblast communication pattern, especially its role as sensory cells.

CONCLUSION

There are several findings indicating the involvement of Ca V1.2 and glutamate canals in odontoblast in the mechanism of sensors delivery. In addition to TRP, Ca V1.2 acts as an entry channel for Ca\(^{2+}\) ions in odontoblasts, followed by glutamate release, which is characterized by mGluRs, so odontoblasts can communicate paracrine both interodontoblasts and odontoblasts-TG in response to tooth pain.

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