International Journal of Innovative Computing, Information and Control
Volume **17**, Number **6**, December **2021**

ICIC International ©2021 ISSN 1349-4198
pp. **1989–2017**

MODELING OF THE VASORELAXATION MECHANISM BY ACUPUNCTURE/MOXIBUSTION STIMULATION FOR PREVENTING ARTERIOSCLEROSIS –REVIEW PAPER–

Tetsuya Yoneda[1], Takeshi Yamakawa[2] and Chikamune Wada[1]

[1]Graduate School of Life Science and Systems Engineering
Kyushu Institute of Technology
2-4 Hibikino, Wakamatsu-ku, Kitakyushu-shi, Fukuoka 808-0196, Japan
yoneda.tetsuya677@mail.kyutech.jp; wada@brain.kyutech.ac.jp

[2]Fuzzy Logic Systems Institute
1-5-204 Hibikino, Wakamatsu-ku, Kitakyushu-shi, Fukuoka 808-0135, Japan
yamakawa@flsi.or.jp

Received February 2021; revised June 2021

Abstract. *Arteriosclerosis requires early detection and treatment. However, its treatment remains insufficiently established. Elucidating the mechanisms of vasorelaxation may lead to early treatment and contribute to the prevention of diseases caused by arteriosclerosis. The ability of acupuncture/moxibustion stimulation to temporarily increase vasorelaxation and vascular flow has been extensively reported, but its mechanism remains unknown. We successfully modeled the mechanism starting from acupuncture/moxibustion stimulation to vasorelaxation/vasodilation by collating the results of numerous studies in various fields, such as medicine, biology, neurosciences, and pharmacology. Ion channels, including transient receptor potential (TRP) channels, exist at free nerve endings. Acupuncture/moxibustion stimulation is sensed by its ion channels and generates nitric oxide in three subsequent pathways, which leads to vasorelaxation. The three pathways are 1) the nNOS activity pathway (based on neural information), 2) eNOS activity pathway (based on vascular wall shear stress), and 3) iNOS activity pathway (based on multiple cytokines produced by macrophages). These pathways differ depending on the external stimulus; however, in each case, they lead to vasorelaxation and contribute to arteriosclerosis prevention. These pathways can be expected to create complex circuits and produce sustained stimulation effects. This model is expected to contribute to the expansion of the research fields and improvement of treatment methods for medical professionals.*

Keywords: Arteriosclerosis, Nitric oxide, Acupuncture, Moxibustion, Vasorelaxation, Vasodilation, Neuronal nitric oxide synthase (nNOS), Endothelial nitric oxide synthase (eNOS), Inducible nitric oxide synthase (iNOS), Ion channel, Transient receptor potential channel (TRP)

1. **Introduction.** According to the World Health Organization (WHO)'s Global Health Estimates 2016 Summary Tables, 15.2 million (26.8%) of the 56.9 million deaths worldwide were caused by ischemic heart disease and stroke, which are then considered as the major causes of death [1]. These diseases are largely attributed to arteriosclerosis and have been the leading cause of death worldwide for the past 15 years. Given that arteriosclerosis has almost no early symptoms and progresses quietly, it is regarded as a "silent disease". Hence, early detection and treatment of arteriosclerosis is necessary, thereby implying timely examination and prevention. Early prevention can be achieved by vasorelaxation

DOI: 10.24507/ijicic.17.06.1989

and increased blood flow. One of the methods for increasing vasorelaxation and blood flow is acupuncture/moxibustion treatment.

Acupuncture/moxibustion is one of the treatments used in traditional Chinese medicine and dates back thousands of years. It involves a technique that promotes vasorelaxation, vasodilation, and blood flow increase, but the mechanism has not been elucidated yet. Knowing the mechanism of vasorelaxation and vasodilation via acupuncture/moxibustion stimulation is crucial not only for acupuncturists but also for physicians, physical therapists, occupational therapists, and other medical professionals.

Vasorelaxation has various causes, but the main cause is NO occurrence. In 1998, Robert Francis Furchgott, Louis J. Ignarro, and Ferid Murad received the Nobel Prize in Physiology or Medicine for their discoveries concerning "nitric oxide (NO) as a signaling molecule in the cardiovascular system". Consequently, NO has attracted research attention. Nitric oxide synthase (NOS) is involved in generating NO and consists of three types as follows [2-8]: neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). These isoforms will be referred to by the most common nomenclature: nNOS (also known as Type I, NOS-I and NOS-1) being the isoform first found (and predominating) in neuronal tissue, iNOS (also known as Type II, NOS-II and NOS-2) being the isoform which is inducible in a wide range of cells and tissues and eNOS (also known as Type III, NOS-III and NOS-3) being the isoform first found in vascular endothelial cells [2]. The three isoforms differ in their dependence on Ca^{2+} as well as in their expressions and activities. eNOS and nNOS are activated by an increase in intracellular Ca^{2+} followed by Ca^{2+}/Calmodulin (CaM) binding. By contrast, iNOS contains irreversibly bound CaM and is therefore largely independent of Ca^{2+}. The only major difference in the reaction between NOS isoforms is the nicotinamide adenine dinucleotide phosphate (NADPH) oxidation rate, where nNOS activity is much higher than eNOS or iNOS [3]. While nNOS and eNOS isoforms constitutively exist in various cell types, such as the endothelium, platelets, and neurons, iNOS is generally induced by cytokines on injury [4, 5]. Nitric oxide, generated by NOS, activates soluble guanylate cyclase (sGC) and particulate guanylate cyclase, and inhibits cytochrome c oxidase. cGMP activates cGMP-dependent protein kinases (PKG) [6]. eNOS, which maintains vasodilation, controls blood pressure and has many other vasoprotective and anti-atherosclerosis effects [7]. NO is produced in the active site of the enzyme in two distinct cycles from oxidation of the substrate L-arg (L-arginine) in NADPH dependent reaction [8].

Acupuncture/moxibustion stimuli include mechanical, noxious, and thermal stimuli. In this study, we review the role of NOS in stimulus sensation, pathway conduction, and NO generation when the body receives stimuli. Moreover, we discuss our attempt to model the mechanism involved during vasorelaxation and vasodilation.

Many reports have demonstrated that acupuncture/moxibustion stimulation increases vascular flow. To give examples, for acupuncture stimulation techniques, the needling intensity is important and DeQi (special sensations and reactions after the insertion of needles) stimulation produces the most significant increase in blood flow in both the skin and muscle [9]. Acupuncture stimulation and phototherapy increase the diameter and blood flow velocity of peripheral small arteries [10], whereas electroacupuncture increases blood flow [11]. Xu et al. determined the blood perfusion rate after treatment with moxibustion and detected a rapid and sudden increase in body temperature [12]. Low-frequency transcutaneous electrical nerve stimulation (TENS) is more effective for increasing peripheral blood circulation than stimulation at acupuncture points [13]. Acupuncture treatment in the auricular region increases cortical regional cerebral blood flow [14]. No general report has described the mechanism of the blood flow increase.

A thorough understanding of the mechanism can immensely contribute to health promotion by increasing the blood flow through acupuncture/moxibustion. More importantly, it may contribute significantly to the treatment of arteriosclerosis and prevention of many arteriosclerosis-induced diseases such as angina, myocardial infarction, and cerebral infarction.

Thus far, no studies have elucidated the mechanism that leads to vasorelaxation by acupuncture/moxibustion stimulation. Therefore, we surveyed and correlated existing studies in various academic fields, such as medicine, biology, neuroscience, and pharmacology. Our final goal was to model the mechanism of vasorelaxation to prove the effectiveness of acupuncture/moxibustion stimulation. Therefore, we hypothesized the process from acupuncture/moxibustion stimulation to vasorelaxation and modeled the mechanism of vasorelaxation by collating the findings from existing studies. Acupuncture/moxibustion stimuli are sensed by receptors at free nerve endings, and depending on the type of stimulus, they take several routes of nNOS, eNOS, and iNOS, leading to relaxation of smooth muscle in blood vessels.

This paper comprises the following sections: 2. Basic concepts for understanding this paper, 3. Mechanisms of vasorelaxation and vasodilation, 4. Proposal of a vasorelaxation model by acupuncture stimulation, 5. Discussion, and 6. Conclusion.

2. Basic Concepts for Understanding This Paper.

2.1. Stimulation by acupuncture/moxibustion.

Acupuncture/moxibustion is based on the classical theory of traditional Chinese medicine and has developed uniquely in China, South Korea, and Japan. Physical/chemical stimulation such as acupuncture/moxibustion affects various body functions (e.g., endogenous pain suppression system, autonomic nervous system, endocrine system, and immune system) when the acupuncture/moxibustion points, called acupuncture points, are targeted in the skin and muscles via the central nervous system. Acupuncture/moxibustion also enhances the body's natural healing power. In other words, acupuncture/moxibustion therapy utilizes this natural healing power.

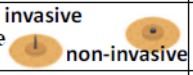
Many treatment points in the human body are used in acupuncture/moxibustion, and the WHO has approved 361 acupuncture points [15] and identified the following diseases for which acupuncture/moxibustion therapy are effective [16]: diseases of the nervous system, motor system, cardiovascular system, respiratory system, digestive system, metabolic endocrine system, reproductive/urinary system, gynecological system, ophthalmic system, and otolaryngological system, and those in pediatric patients.

Stimuli by acupuncture/moxibustion include noxious, mechanical, and thermal/chemical stimuli [17]. The typical tools used in acupuncture/moxibustion treatment include the filiform needle, spoon needle, ring-headed thumbtack needle, and moxibustion, each of which provides a different stimulus for a different purpose. For example, the filiform, spoon, and ring-headed thumbtack needles are applied for mechanical stimuli; the filiform needle, ring-headed thumbtack needle, and moxibustion free type are used for noxious stimuli; and the filiform needle, moxibustion pedestal type, and moxibustion free type are used for hot stimuli. Table 1 shows the relationship between the acupuncture/moxibustion tools and stimulations.

2.2. Arteriosclerosis.

Arteriosclerosis is a condition in which blood vessels become narrower, stiffer, and less flexible. The progression of arteriosclerosis is not constant; it progresses slowly and quietly in the early stage but suddenly develops at an accelerating rate [18]. In arteriosclerotic blood vessels, vascular endothelial function decreases owing to

TABLE 1. Sensations caused by acupuncture/moxibustion stimulations

Acupuncture/Moxibustion Stimulation Types	Nociceptive Sensation	Pressure Sensation	Heat Sensation
Filiform needle	○	○	○
Spoon needle		○	
Ring-headed thumbtack needle 	○	○	
Moxibustion Pedestal type 			○
Moxibustion Free type 	○		○

oxidized low-density lipoprotein (LDL), NO production decreases, vasorelaxation worsens, platelet aggregation increases, and vascular smooth muscle (VSM) cell proliferation increases.

Arteries have three layers: the tunica intima, tunica media, and tunica externa. Moreover, arteriosclerosis can be divided into the following three types: 1) atherosclerosis, 2) Mönckeberg's medial sclerosis, and 3) fine arteriosclerosis, depending on how and where it occurs. These three types of arteriosclerosis are explained as follows.

1) Atherosclerosis. When endothelial cells are damaged by hypertension or diabetes, LDL from the blood enters the intima and is converted into oxidized LDL. As a response, monocytes turn into macrophages. The oxidized LDL undergoes phagocytosis, causing the macrophages to die and be deposited as foam cells or plaques. This plaque formation is called atherosclerosis. These plaques could also suddenly rupture and clot blood in blood vessels, forming blood clots that block the lumen of arteries. Otherwise, the blood clots rupture and clog small arteries, thereby blocking the blood flow and eventually impeding the transport of oxygen and nutrients to organs. Nitric oxide (NO), which will be discussed later, inhibits platelet aggregation and acts as an anti-atherosclerotic agent [19-22].

2) Mönckeberg's medial sclerosis. The tunica media of the arteries comprises smooth muscle and elastic fibers. In Mönckeberg's arteriosclerosis, calcium accumulates in the tunica media of the artery and becomes ossified, resulting in the loss of elasticity. As it progresses, the tunica media become stiff and brittle, leading to vessel rupture. Common areas affected are the aorta, lower limb arteries, and cervical arteries. Furthermore, it is often found in men and women over 50 years old. This type is expected to improve by the action of NO, which relaxes VSM [7, 22].

3) Fine arteriosclerosis. In fine arteriosclerosis, the arterial blood vessels become nonelastic and hard because of blood vessel wall aging. Considering its lack of elasticity, it is prone to blood vessel rupture when the blood pressure is high. This rupture is particularly dangerous because it can lead to stroke, which causes sudden paralysis of the body's functions if the rupture occurs in the brain. The known definitive solution is to administer drugs that lower blood pressure. However, improvement is expected with the action of NO, which relaxes VSM [7, 22].

2.3. **Cell surface receptor.** Cell surface receptors are classified into four types: 1) ion channel receptors, 2) transient receptor potential (TRP) channel, 3) G protein-coupled receptors (GPCRs), and 4) enzyme-linked receptors. Each of the four types is described as follows.

1) Ion channel receptor. Ion channels are membrane proteins present in the plasma membrane or the endomembrane system. The lipid bilayer membrane, hardly permeates ions. Nevertheless, ion channels, which are expressed in all cells from bacteria to higher animals, allow ions to permeate inside and outside the membrane. The type or size of ions that can pass through is determined by the ion selectivity filter existing in the ion transmission path. Ions flow through pores, with gates along the way that, if open, identify the charge and size of the ions, thereby allowing only specific ions to pass. Typical ion channel receptors are nicotinic acetylcholine receptors, ion channel glutamate receptors N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, and gamma-aminobutyric acid receptors. In this review, NMDA receptors, AMPA receptors, TRP channels are related.

2) TRP channel. TRP channels are nonselective cation channels originally found in Drosophila. The mammalian TRP superfamily has 28 cation permeable channels subdivided into six subfamilies [23-25]. The TRP channel has a six-transmembrane structure formed by a tetramer, and it functions as an ion channel. Activated openings of TRP channels are triggered by various physicochemical stimuli, such as temperature, mechanical stimuli, pain, and acid-base balance, and many exhibit high Na^+ and Ca^{2+} permeability. In particular, TRPV is activated by stimuli such as mechanical stimuli, temperature rises, pH changes, chemical substances such as NO, and osmotic pressure changes [26]. TRP channels at free nerve endings [17] sense acupuncture/moxibustion stimuli, leading to vasorelaxation.

3) GPCRs. GPCR exists in body tissues and cells. Some GPCRs have various functions, forming the largest superfamily of known proteins, with more than 800 currently identified [27-30]. GPCRs, which are also called seven-(pass)-transmembrane domain receptors, transmit extracellular chemical information into the cell. Most drugs act on GPCRs to exert their effects. Signal transmission is via G proteins that are coupled to GPCRs. GPCRs are widely involved in maintaining homeostasis in living organisms. GPCRs at free nerve endings receive endogenous algogenic substances, such as histamine and bradykinin, which are secreted by acupuncture/moxibustion stimulation [31, 32]. As a result, vasorelaxation occurs.

4) Enzyme-linked receptor. Enzyme-linked receptors activate either the enzyme itself or a related enzyme directly. These receptors are usually single transmembrane receptors, and the enzymatic component of the receptor is retained in the cell. The majority of enzyme-linked receptors are protein kinases or bind to protein kinases.

2.4. **Primary afferent nociceptors.** Primary afferent nociceptors include A-delta (Aδ) and C fibers. Aδ fibers are thick (1-5 μm in diameter) myelinated fibers with a high transmission rate (5-30 m/s), and they mainly transmit tactile, pain (sharp pain), and temperature sensations. Meanwhile, C fibers are thin (0.2-1.5 μm in diameter) unmyelinated fibers with a slow transmission rate (0.5-2 m/s), and they mainly transmit dull pain, temperature, and itching sensations [33]. At the free nerve endings, "noxious receptors" such as high-threshold mechanoreceptors and polymodal receptors exist as pain sensation receptors.

High-threshold mechanoreceptors respond only to high-threshold mechanical stimuli, such as pressure, cutting, and stabbing, which are harmful to the organism, and they do not respond to thermal and chemical stimuli. Polymodal receptors, as the name implies, propagate various types of information to the center; they respond to all low- to high-threshold mechanical, thermal, and various noxious chemical stimuli.

Notably, when noxious stimuli are repeated at the same site (receptive field) with the same intensity, the polymodal receptors lower the threshold value, increase responsiveness to the stimulus, expand the receptive field, and increase spontaneous discharge. These phenomena are collectively called sensitization. Almost exclusively, polymodal receptors are present in the free nerve endings of fibers [34, 35]. The acupuncture stimulus is received by the receptors, causing an influx of ions, depolarization, and impulse generation, which is transmitted via the primary afferent neurons.

2.5. **NMDA receptor.** NMDA receptor is a type of glutamate receptor that plays an important role in memory and learning in the hippocampus, and is deeply involved in neuronal cell death after cerebral ischemia. NMDA receptors are present in the skin, peripheral sensory axons, endothelium, kidneys, and bones [36-40].

Moreover, NMDA receptor is selective for NMDA as an agonist. NMDA receptors are generally blocked and inactivated by Mg^{2+} when the membrane potential is largely negative, but unblocked when the membrane potential is positive or between -10 to -20 mV. Conversely, NMDA receptors are activated when glutamate stimulation from presynaptic terminals and depolarization of the postsynaptic membrane occur concurrently, causing Ca^{2+} influx from the postsynaptic membrane [41, 42].

Ion channel glutamate receptors consist of three types, namely, AMPA receptors, NMDA receptors, and kainate receptors [43, 44].

2.6. **Vascular endothelial shear stress.** Shear stress is a type of stress that acts to slide on a certain surface inside the object in the direction parallel to the surface. The vascular wall is constantly subjected to hemodynamic stresses such as blood pressure forces acting perpendicular to the vessel wall (stretch) and blood flow forces acting tangentially to the vessel lumen surface (wall shear stress). Shear stress is a physical force proportional to blood viscosity and blood flow's velocity gradient, which acts only on the vascular endothelium and distorts the endothelial cells in the direction of blood flow. This phenomenon stimulates the alteration of endothelial cell function. The endothelium is subjected to a shear stress of approximately 20 dynes/cm^2 in the arteries and 1.5-6 dynes/cm^2 in the veins. When stenosis occurs in the coronary arteries, the blood flow velocity in that area increases, and the shear stress may reach 500 dynes/cm^2. Shear stress is relatively low in the arterial bends and bifurcations that are common in atherosclerotic lesions, and the blood flow is turbulent and unsteady in its direction and strength. Furthermore, shear stress is directly related to blood flow, and it is proportional to blood flow speed but inversely proportional to the cube of the vessel radius [45]. In this review, we demonstrate that this endothelial shear stress occurs in the eNOS activation pathway.

2.7. **Leukocyte migration from blood vessels.** Leukocytes, which are immunocompetent cells, constantly circulate in blood vessels. Cell migration is the movement of cells from one place to another in the body. Leukocytes migrate to infiltrate the inflamed extravascular tissue. Among the leukocytes, neutrophils freely pass through blood vessel walls while deforming themselves to phagocytose the invading bacteria. This mechanism is called extravascular migration. Approximately 30-60 minutes after tissue damage, leukocytes such as neutrophils, macrophages, and lymphocytes gather at the inflammation site [46-48]. Then, these leukocytes do not exit from the arteries and capillaries at the

inflammation site but from the small veins leading to the capillaries [49]. Among them, macrophages respond to bradykinin and other peptides, releasing inflammatory cytokines such as interleukins (ILs), and tumor necrosis factor-α (TNF-α) [50]. These inflammatory cytokines also have a pain-enhancing effect, which also stimulates polymodal receptors and intensifies pain. Nociceptive stimulation by acupuncture/moxibustion induces the migration of leukocytes from blood vessels [46-48].

3. **Mechanisms of Vasorelaxation and Vasodilation.** This section describes the general mechanisms of the contraction and relaxation of VSMs; vasorelaxation by NO; generation of NO by nNOS, eNOS, and iNOS; and relaxation of VSM by NO as described earlier.

3.1. **Mechanisms of VSM contraction and relaxation.** Similar to the arteries, the arteriovenous vessel wall consists of three layers, tunica intima, tunica media, and tunica externa. The tunica intima is composed of a single layer of endothelial cells and a small amount of connective tissue. The tunica media is composed of VSMs and elastic fibers that run in a ring. The larger the artery, the more elastic and flexible fibers are developed. The tunica media in veins is thinner, with fewer elastic fibers, than that in arteries.

Smooth muscle is mainly responsible for the contraction of blood vessels and the digestive tract, and it contracts and relaxes by a mechanism different from that of skeletal muscle. In smooth muscles, depolarization and mechanical stimulation of the cell membrane open Ca^{2+} channels in the plasma membrane and allow Ca^{2+} to flow into the cytoplasm from outside the cell. The inflow of Ca^{2+} triggers the release of Ca^{2+} from the sarcoplasmic reticulum. The released Ca^{2+} binds to CaM, activates myosin light chain kinase (MLCK), and promotes phosphorylation of the myosin light chain (MLC). MLC phosphorylation causes muscle contraction because of the interaction between actin and myosin. Regarding relaxation, cyclic guanosine monophosphate (cGMP) in VSM activates protein kinase G (PKG), and PKG activates myosin light chain phosphatase (MLCPh). PKG also lowers the intracellular calcium concentration. The activation of MLCPh promotes MLC dephosphorylation, which then causes muscle relaxation. When MLC phosphorylation is reduced, the VSM relaxes.

3.2. **Mechanism of NO-induced vasorelaxation.** Endothelium-derived relaxing factors such as prostacyclin and NO, and endothelium-derived hyperpolarizing factor (EDHF) are substances that affect vasorelaxation. In addition, carbon dioxide, serotonin, and histamine induce vasorelaxation. This review discusses the mechanism of vasorelaxation by NO. The vascular endothelium relaxes the surrounding smooth muscles by triggering NO generation, and the pressure within the vessel causes the artery to dilate and increases blood flow. Hence, nitrite derivatives such as nitroglycerin, amyl nitrite, and isosorbide mononitrate are used to treat heart disease. These compounds convert into NO, which increases blood supply to the coronary artery through dilation.

NO binds to intracellular soluble guanylate cyclase (sGC) and promotes the synthesis of cGMP [51, 52]. The synthesized cGMP activates cGMP-dependent protein kinase, particularly PKG [53-55]. Consequently, the intracellular influx of Ca^{2+} involved in smooth muscle contraction is suppressed, leading to relaxation of blood vessels. Dissociation of the Ca^{2+}/CaM complex and decrease in intracellular Ca^{2+} levels inactivate MLCK, and relax the smooth muscle.

3.3. **Mechanism of nNOS-induced vasorelaxation.** Figure 1 shows the mechanism of nNOS-induced vasorelaxation. nNOS is mainly expressed in neurons found in the central and peripheral nervous systems. Postsynaptic density (PSD) is an efficient coupling

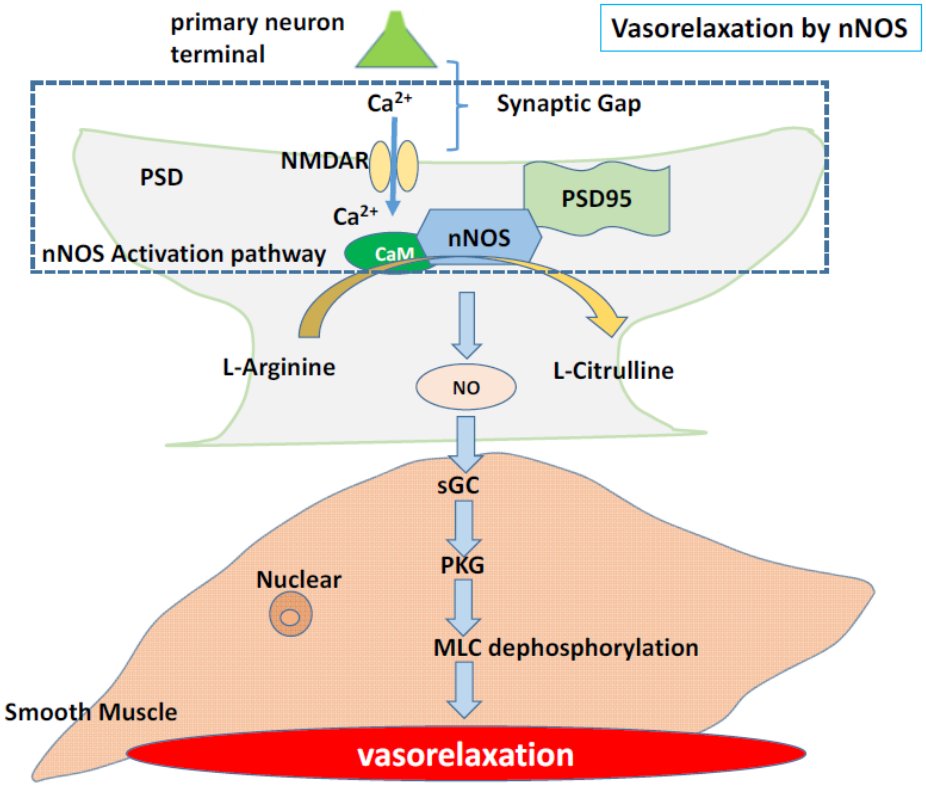


FIGURE 1. Mechanism of neuronal nitric oxide synthase (nNOS)-induced vasorelaxation. When Ca^{2+} is transported to inside of the postsynaptic cell from the NMDA receptor (NMDAR), nNOS is activated and acts as an enzyme to synthesize NO. VSM relaxes when the produced NO diffuses into the smooth muscle.

of nNOS and NMDA receptors via PSD-95. Moreover, nNOS is activated by Ca^{2+}/CaM [56, 57] and Ca^{2+} entering through NMDA receptors. In the vascular endothelium, nNOS causes a reaction between L-arginine and oxygen, leading to the production and diffusion of NO [2, 58].

NO synthesized by nNOS is released from nerve endings and activates sGC in VSM and increases cGMP concentration. cGMP activates cGMP-dependent protein kinase (PKG) [59-61] and relaxes blood vessels. Considering that nNOS is present in the nervous system, it produces relatively large amounts of NO through cascading activation by NMDA receptor stimulation.

3.4. **Mechanism of eNOS-induced vasorelaxation.** Figure 2 shows the mechanism of eNOS-induced vasorelaxation. eNOS binds to caveolin-1, a major membrane protein that forms intracellular structures called caveolae [62, 63]. In response to shear stress changes on the vascular wall, eNOS localized in the caveolae, a specialized region in cell membranes, increases Ca^{2+} in vascular endothelial cells. Ca^{2+}/CaM activates eNOS and releases NO from L-arginine [3]. For instance, when blood flow increases by performing light exercise, shear stress escalates, followed by increased NO production from vascular endothelial cells.

3.5. **Mechanism of vasorelaxation by iNOS.** Figure 3 shows the mechanism of vasorelaxation by iNOS. Monocytes, one of the leukocytes, migrate to extravascular tissues

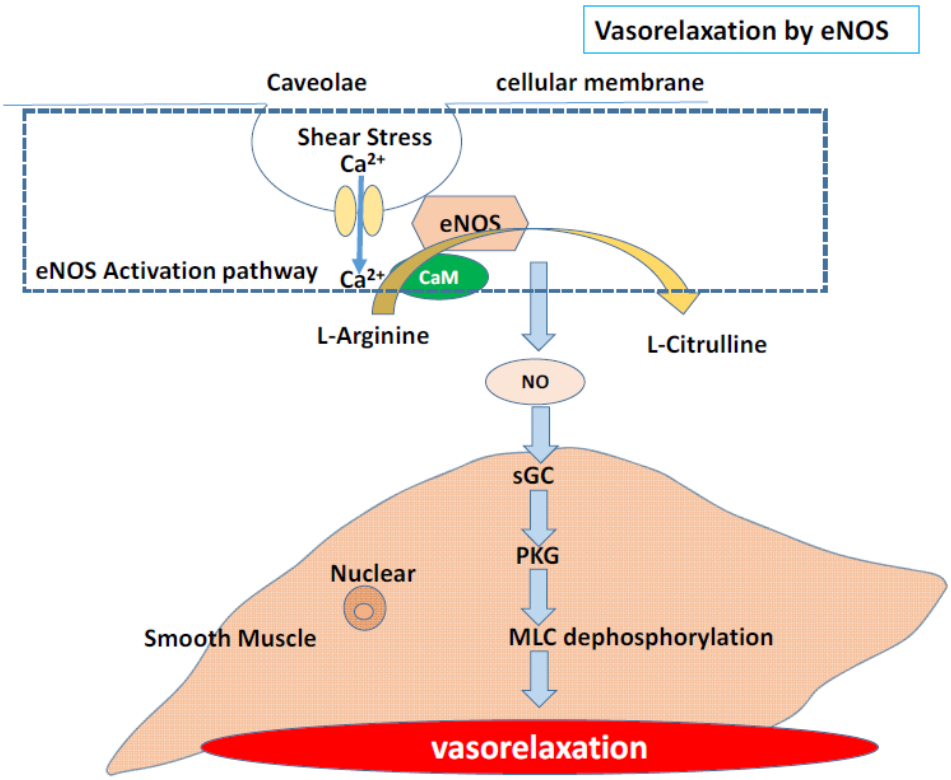


FIGURE 2. Mechanism of endothelial nitric oxide synthase (eNOS)-induced vasorelaxation. When shear stress occurs in the vascular endothelium owing to increased blood flow, eNOS is activated and acts as an enzyme to synthesize NO. VSM relaxes when the produced NO diffuses into the smooth muscle.

and body cavities, where they differentiate into tissue-specific macrophages. Lipopolysaccharide (LPS) and interferon (IFN) stimulate macrophages to produce inflammatory cytokines via toll-like receptors on the cell surface, leading to the induction of iNOS [64-66] and production of NO.

iNOS is always in an active state because of the very strong binding of CaM to iNOS and its high Ca^{2+} affinity. Macrophage, produces NO to kill pathogens. However, macrophages can also cause an adverse effect. In sepsis, macrophages produce large amounts of NO, and the resulting vasodilation is the main cause of hypotension. In a normal state, the induced gene expression of iNOS is suppressed at low level, but is induced by cytokine stimuli such as lipopolysaccharide and IFN-γ [7, 65, 67], causing iNOS to produce a considerable amount of NO.

4. **Proposal of a Vasorelaxation Model by Acupuncture/Moxibustion Stimulation.** In acupuncture/moxibustion stimulation, NOS acts as an enzyme via three pathways which are nNOS, eNOS, and iNOS activation pathways, and synthesizes L-citrulline and NO from L-arginine [58, 68, 69]. When the produced NO diffuses into the smooth muscle, VSM relaxes [53-55, 70] (Figure 4).

Depending on the type of acupuncture/moxibustion stimulus, the conduction of the stimulus may travel via the nNOS, eNOS, or iNOS activation pathways with a time lag [3] to generate NO and vasorelaxation [71-73]. NO generation is higher in the order of iNOS $\gg$ nNOS $>$ eNOS [74, 75]. Figure 5 shows the overall flow diagram of vasorelaxation by acupuncture/moxibustion stimulation.

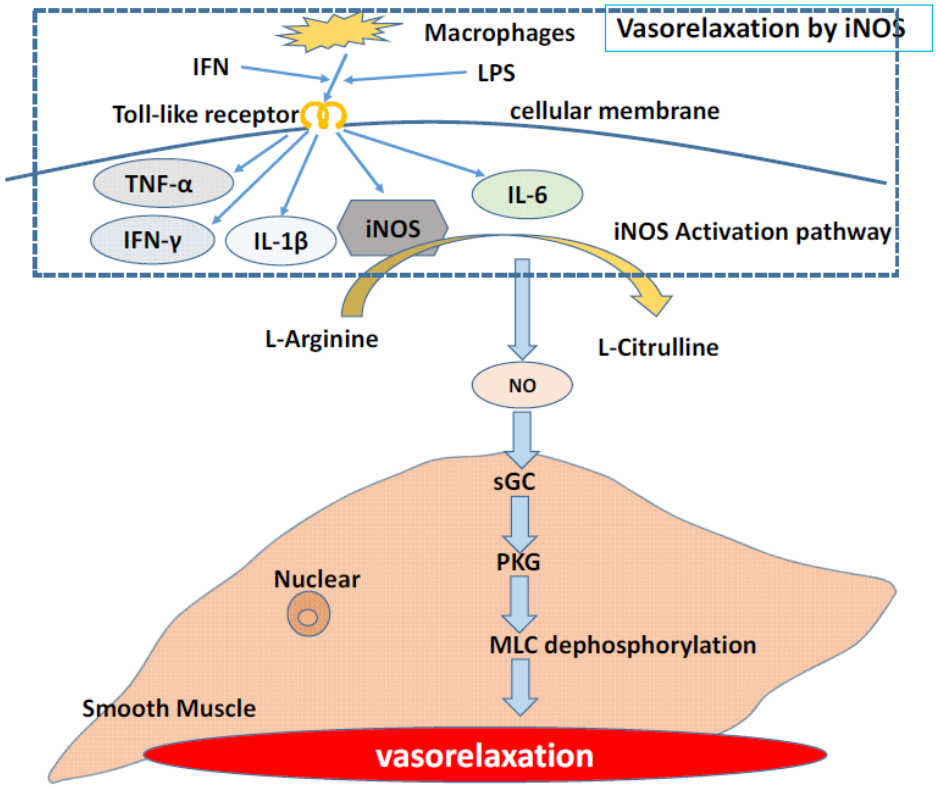


FIGURE 3. Mechanism of vasorelaxation by inducible nitric oxide synthase (iNOS). Lipopolysaccharide (LPS) and interferon (IFN) stimulate macrophages, and iNOS is induced via toll-like receptors on the cell surface. iNOS is activated by the produced cytokines, namely, TNF-α, IFN-γ, IL-1β, and IL-6, which then acts as an enzyme and synthesizes NO. VSM relaxes when the produced NO diffuses into the smooth muscle.

nNOS and eNOS are calcium dependent and produce low levels of NO as cellular signaling molecules. On the other hand, iNOS is calcium-independent [4, 56, 58] but produces large amounts of NO and is cytotoxic [67, 76, 77].

The following TRP and ion channels are typical examples that act as nociceptors that sense acupuncture/moxibustion stimuli. The nociceptors piezo1/2, TRPV1, TRPV4, and ASICs sense mechanical stimuli [78-82], whereas TRPV1-TRPV4 sense thermal stimuli [83]. TRPV1 is activated at $\geq$ 43°C; TRPV2, at $\geq$ 53°C; TRPV3, at 34-38°C; and TRPV4, at 27-35°C [83]. In addition, TRPM8 and TRPA1 are activated by cold stimuli; ASICs, TRPV1, TRPV3, TRPM8, and TRPA1, by chemical stimuli [84]; and ASIC channels, by pH changes. Table 2 shows the relationship between NOS and receptors that receive acupuncture/moxibustion stimuli.

4.1. **Vasorelaxation by nNOS activation pathway.** Figure 6 shows a flowchart of vasorelaxation by the nNOS activation pathway. When the receptor at the free nerve terminal senses a stimulus, an ion channel opens, allowing extracellular Ca^{2+} to flow into the cell. When the depolarized membrane potential reaches the threshold, Na^+ channel opens, causing a large amount of Na^+ to flow into the cell. Consequently, the potentials inside and outside the cell are reversed, and active potentials are generated. Adjacent Na^+ channels open one after another, and nerve impulses run along the nerve toward the spinal cord [85, 86].

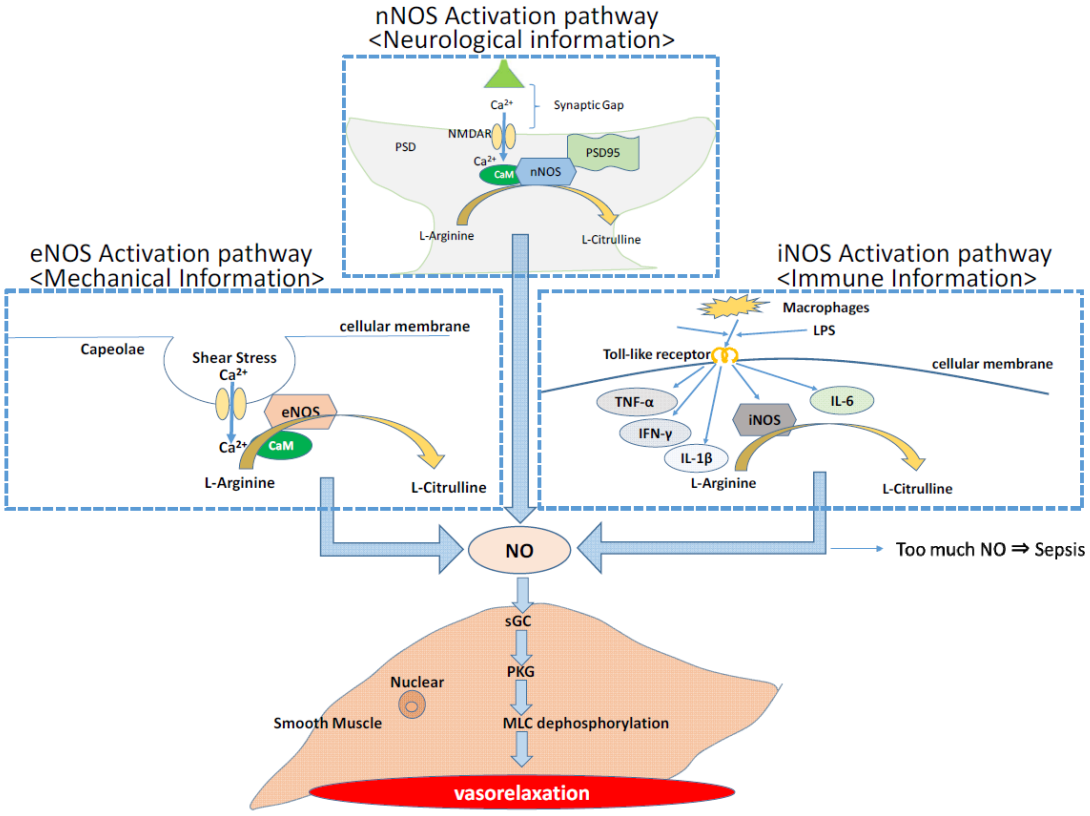


FIGURE 4. Vasorelaxation model by acupuncture/moxibustion stimulation. Acupuncture/moxibustion stimulation synthesizes L-citrulline (L-Cit) and NO from L-arginine (L-Arg) via three pathways, namely, the nNOS, eNOS, and iNOS activation pathways. Vasorelaxation occurs when the produced NO diffuses into the smooth muscle.

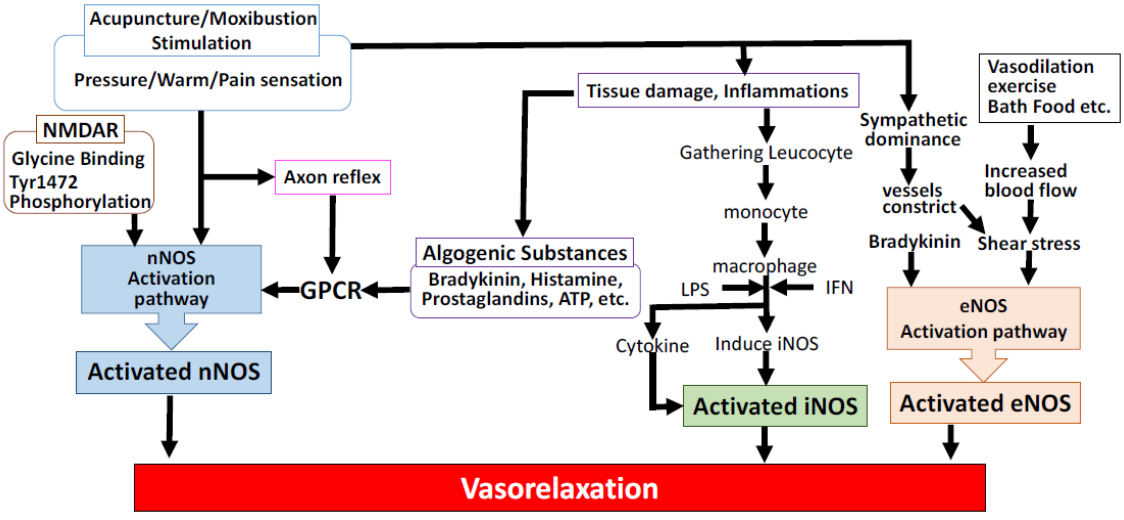


FIGURE 5. Overall flow diagram of vasorelaxation by acupuncture/moxibustion stimulation. Acupuncture/moxibustion stimuli lead to vasorelaxation in all the cases, with different pathways, time delays, and feedback loops selected depending on the type of stimulus. It can be inferred that this creates a complex circuit and produces a sustained effect of the stimulus.

TABLE 2. Acupuncture/moxibustion stimulation and nitric oxide synthase (NOS). nNOS is activated by nociceptive, mechanical, and thermal stimuli; eNOS by mechanical stimuli; and iNOS by nociceptive and thermal stimuli.

	nNOS	eNOS	iNOS
Aggressive Chemical Stimulation	○ [ASICs, TRPV1 TRPV3, TRPM8 TRPA1]		○
Mechanical Stimulation	○ [piezo1/2 TRPV1, TRPV4 ASICS]	○	
Aggressive Thermal Stimulation	○ [TRPV1, TRPV2 TRPV3, TRPV4]		○

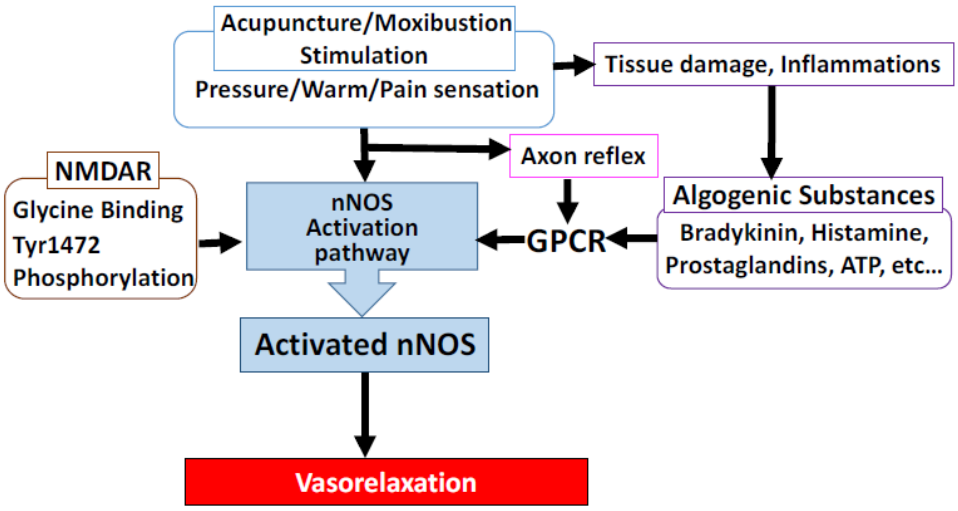


FIGURE 6. Vasorelaxation by the nNOS activation pathway. Sensitive to acupuncture/moxibustion stimulation, the nNOS activation pathway is activated by orthodromic conduction, axon reflex, algogenic substances, pain-enhancing substances, and others, resulting in vasorelaxation.

Conduction consists of two types, orthodromic conduction to the spinal cord and brain and antidromic conduction (axon reflex), which is retrograde at axonal bifurcation.

In addition, as a meridian that activates nNOS, nociceptive stimuli generate algogenic substances and pain-enhancing substances.

4.1.1. *Orthodromic conduction.* Figure 7 shows information transmission at the dorsal horn synapse of the spinal cord. Aδ fibers conduct sharp pain, whereas C fibers do dull pain. When the nerve impulse reaches the end of the primary afferent fiber on the synaptic side, flows into the inside of the ending of neuron through the voltage-gated Ca^{2+} channel and releases synaptic vesicle neurotransmitters: in the case of Aδ fibers, synaptic vesicular neurotransmitters such as glutamate, and in C fibers, glutamate and substance P (SP) of synaptic vesicles are released [87-91].

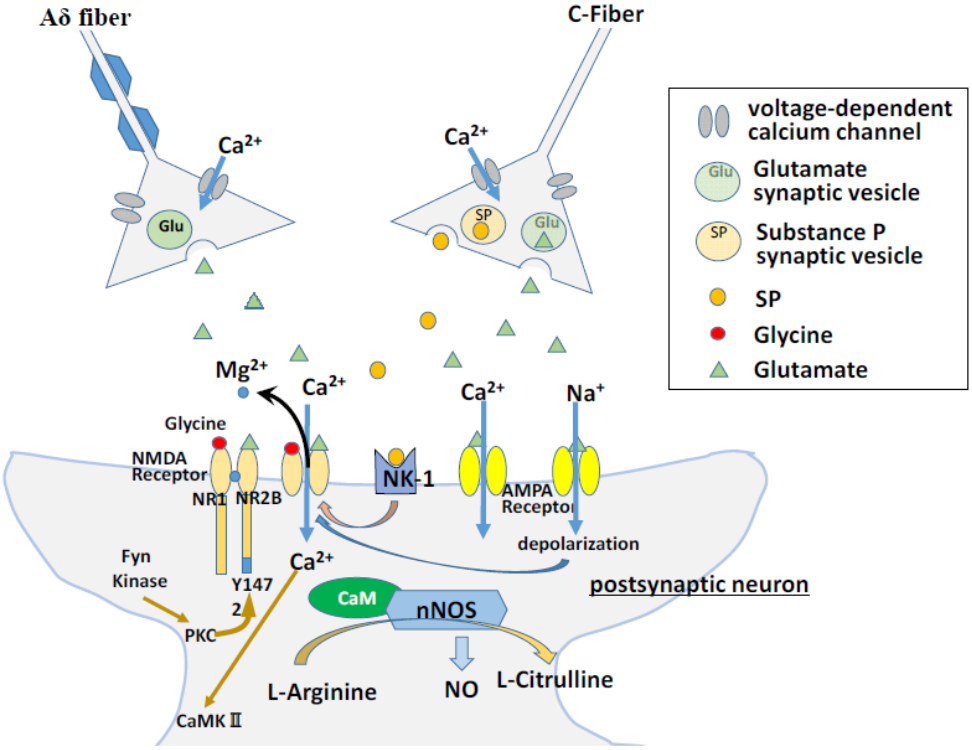


FIGURE 7. Information transmission at the dorsal horn synapse of the spinal cord. Glutamate, SP, and others released from the peripheral terminals of Aδ and C fibers activate the NMDA receptor by the AMPA and NK-1 receptors, which are imbeded in the post synaptic membrane, and blocked Mg^{2+} is released. The influx of Ca^{2+} from the NMDA receptor causes a chain of reactions.

On the secondary neuron side of the synapse, glutamate released from the primary neuron binds to AMPA receptors, which then secrete a large amount of Na^{+} that depolarizes them. This event unblocks the NMDA receptor, which is normally blocked by Mg^{2+} [42, 92, 93]. Removal of Mg^{2+} block involves four auxiliary roles as follows. 1) The released SP from the terminals of primary afferent neurons binds to NK1, which is a GPCR on the side of secondary neurons [94, 95] and contributes to Mg^{2+} unblocking in NMDA receptors. 2) Binding of the nonessential amino acid glycine to NMDA causes NMDA receptor phosphorylation, contributing to the removal of Mg^{2+} block of NMDA receptors [92]. 3) When synaptic glutamate increases, glutamate and glycine bind to NMDA receptors, thereby increasing AMPA-dependent Na^{+} influx, triggering protein kinase phosphorylation of NMDARs, and removing Mg^{2+} block [92]. 4) Fyn kinase (a tyrosine kinase) and prostaglandin activate protein kinase C (PKC) that subsequently phosphorylates Tyr1472, which is the NR2B subunit of the NMDA receptor, and contributes to Mg^{2+} block removal [96-98].

When the Mg^{2+} block in the NMDA receptor is removed, Ca^{2+} flows in from the NMDA receptor. Then, the nNOS localized in the PSD binds to the Ca^{2+}/CaM complex [99, 100].

The Ca^{2+} flow from the NMDA receptor activates PKC and calcium calmodulin-dependent protein kinase II (CaMKII), which are both Ca^{2+}-dependent intracellular signal transduction systems [101-106]. Concurrently, NMDA and AMPA receptors expressed on the extrasynaptic membrane or contained under the postsynaptic membrane migrate to the postsynaptic membrane [104, 107-112]. Consequently, NMDA and AMPA receptors

increase, causing a large influx of Ca^{2+}, followed by synaptic plasticity, specifically long-term potentiation (LTP) [43, 113, 114].

When CaM activates NOS, NO is released from L-arginine. The reaction equation is as follows [115, 116]:

(L-arginine + NADPH + H^+ + O_2 → NOHLA + $NADP^+$ + H_2O NOHLA + 1/2 NADPH + 1/2 H^+ + O_2 → L-citrulline + 1/2 NAD P^+ + NO + H_2O)

Thus, NO is synthesized by NOS, which oxidizes the guanidine nitrogen of L-arginine.

In this reaction, nNOS moves electrons to heme and continues to oxidize NADPH at a high rate; hence, the reaction rate is much faster than those of eNOS and iNOS [3].

When the generated and diffused NO reaches the smooth muscle of blood vessels, cGMP increases [61, 93, 117, 118]. Then, cGMP activates the muscle relaxant PKG. PKG promotes Ca^{2+} uptake into the sarcoplasmic reticulum [119].

4.1.2. *Antidromic conduction.* Figure 8 shows the axon reflex. Antidromic conduction generates an active potential at the end of the primary afferent fiber, and the nerve impulse folds back at the axonal bifurcation, leading to an axon reflex toward the terminal [120]. The peripheral terminals of the Aδ fibers release glutamate, whereas those of C fibers release glutamate, calcitonin gene-related peptides (CGRP), and SP [89, 90, 95, 120, 121]. The released CGRP produces cyclic adenosine monophosphate in VSM, resulting in localized vasorelaxation and vasodilation [122, 123].

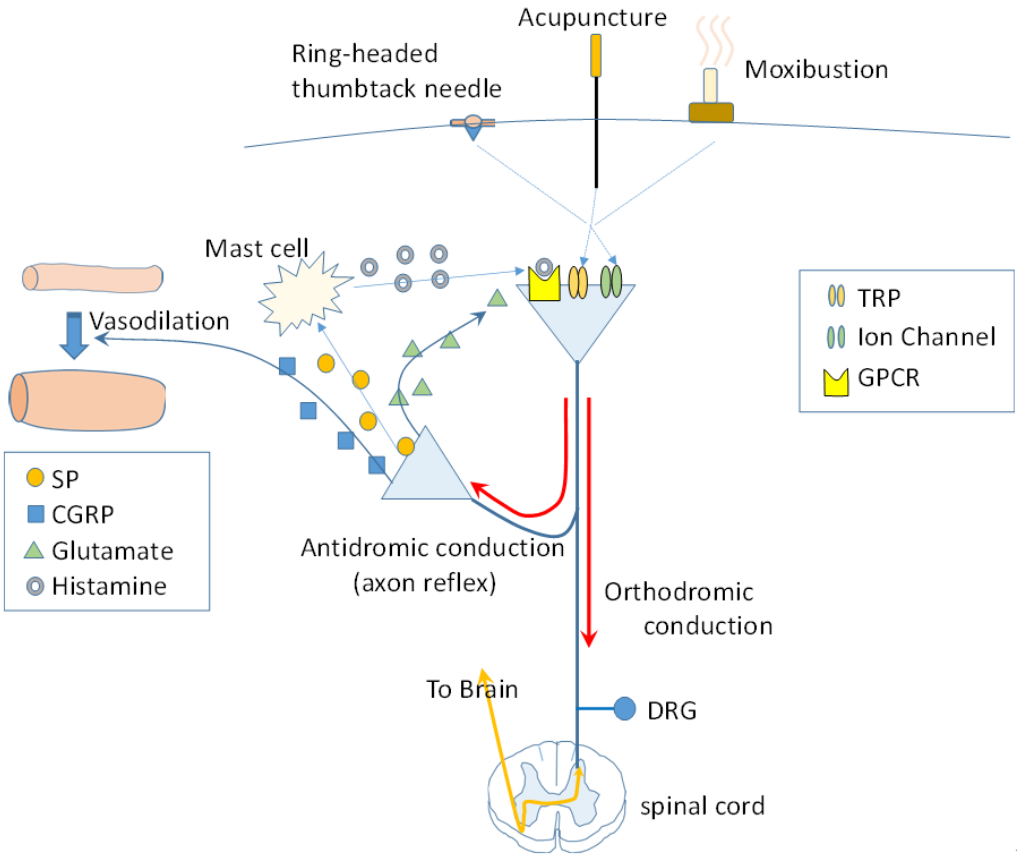


FIGURE 8. Axon reflex. Axon reflexes toward nerve endings occur at the bifurcation of axons, and local vasodilation due to CGRP occurs. By the stimulation of the released SP, histamine and glutamate from mast cells bind to GPCRs at nearby nerve endings and impulses are generated again to form a circulatory pathway.

SP released by axon reflexes binds to NK-1 receptors on mast cells [94, 95, 124] and promotes the release of histamine [124], which binds to GPCRs on nearby free nerve endings, again generating action potentials. Furthermore, neuropeptide SP causes neurological inflammation [125, 126]. Meanwhile, glutamate released by axon reflexes binds to the GPCR (mGluR) of glutamate present at free nerve endings [37], generating additional active potentials. This mechanism also increases the sensitivity of TRP channels and lowers the temperature threshold of TRPV1 from 43°C to 32°C [127-129], enabling the generation of action potentials even at a normal body temperature.

In addition, the generated nerve impulses produce orthodromic conduction to the spinal cord and brain and antidromic conduction of axon reflexes to the opposite endings, creating a repeating circulatory circuit. This glutamate release in the periphery and repeated neurogenic inflammation decrease the threshold for polymodal receptor responses, increased responsiveness, and receptor expansion, which lead to sensitization and LTP [130-133].

4.1.3. *Conduction by algogenic substance and pain-enhancing substance.* Figure 9 shows the generation of algogenic substances and pain-enhancing substances by acupuncture/moxibustion stimulation. In addition to orthodromic and antidromic conductions, some substances activate nNOS because of the generation of algogenic substances and pain-enhancing substances.

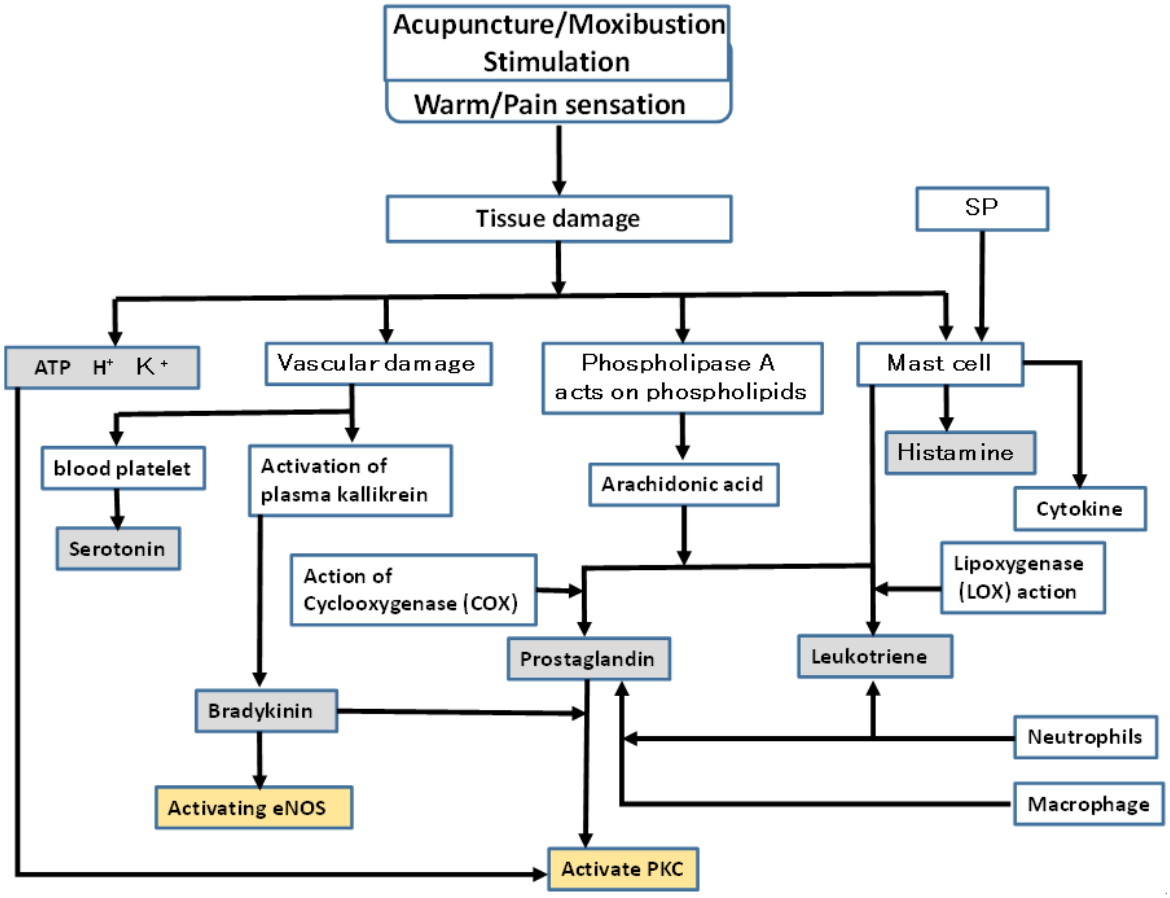


FIGURE 9. Algogenic and pain-enhancing substances generated by acupuncture/moxibustion stimulation. The generated algogenic and pain-enhancing substances bind to GPCRs at free nerve endings, generate impulses, and cause vasorelaxation.

When tissue damage caused by acupuncture/moxibustion stimulation occurs, algogenic substances and pain-enhancing substances such as bradykinin, histamine, prostaglandins, serotonin, leukotrienes, adenosine triphosphate (ATP), H^+, and K^+ are generated [120, 134-136]. Algogenic and pain-enhancing substances bind to GPCRs at free nerve endings [31, 137], generate active potentials, and run nerve impulses toward synapses. By binding the ligands of the algogenic and pain-enhancing substances to each GPCR, the temperature threshold of TRPV1 is lowered from 42°C to 35°C, and active potentials are likely to be generated [127, 129].

Consequently, the impulses that travel to the spinal cord and brain again and those that travel to the nerve endings in an antidromic manner are reproduced, which leads to vasorelaxation and vasodilation. Again, a cyclic circuit is generated. Each algogenic substance and pain-enhancing substance is generated as follows.

Bradykinin is generated by tissue damage that harms blood vessels and activates kallikrein in plasma. It is a proinflammatory peptide, a pain mediator, and a potent vasodilator [138-140]. Meanwhile, histamine is generated by SP and from mast cells caused by tissue damage [141-143], and it dilates blood vessels [144]. Mast cells also release mediators such as leukotrienes and cytokines [145, 146]. Moreover, prostaglandins are produced by phospholipase A acting on phospholipids and arachidonic acid acting on cyclooxygenase (COx) in response to tissue damage [147]. Prostaglandins are also generated by neutrophils and macrophages [148, 149] and have a vasodilatory effect [150]. In addition, serotonin is generated by platelets after tissue damage and vascular injury [151] and has both vasoconstrictor and vasodilator effects [152]. Leukotrienes, such as prostaglandins, are generated from arachidonic acid by the action of lipoxygenase (LOx) [153]. Leukotrienes also have a vasodilatory effect [154]. Likewise, ATP, H^+, and K^+, which are all generated by tissue damage [155], result in vasodilation [156-158].

Unlike the other substances, bradykinin is an endothelium-dependent vasodilator [158, 159].

4.2. **Vasorelaxation by the eNOS activation pathway.** Figure 10 shows the flowchart of vasorelaxation by the eNOS activation pathway. Three pathways activate eNOS for vasorelaxation and vasodilation, which are shear stress, sympathetic nerve, and bradykinin.

1) Vasorelaxation due to the effect of shear stress. Vasorelaxation by the nNOS activation pathway by acupuncture/moxibustion increases the blood flow rate. In addition to acupuncture/moxibustion stimulation, exercise, bathing, and food can increase blood flow. Increased blood flow causes shear stress in the vascular endothelium [55]. Shear stress increases Ca^{2+} concentration [160, 161], and eNOS is activated by binding Cam, subsequently releases NO from L-arginine. This leads to vasorelaxation and vasodilation [68, 162]. Vascular relaxation due to shear stress occurs a little later than nNOS, which reacts quickly [3].

2) Sympathetic vasodilation. Upon receiving acupuncture/moxibustion stimulation, the sympathetic nerve dominates temporarily [163-168]. When blood vessels constrict because of sympathetic dominance, blood flow velocity increases [45]. Increased blood flow causes shear stress at the endocelium cell, Ca^{2+} flows into the cell, and NO is generated in the cell [55, 169], which leads to vasorelaxation. The vasorelaxation is mainly caused by sympathetic stimulation or decreased tension [170].

3) Vasorelaxation with bradykinin. Bradykinin acts on the B2 receptor, which is a GPCR on the endothelial cell membrane, binds to CaM, and increases the intracellular

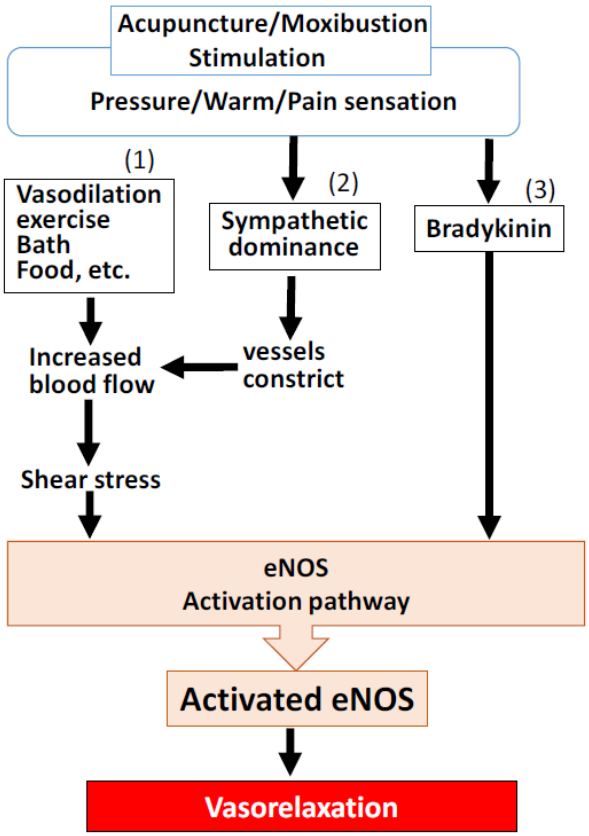


FIGURE 10. Vasorelaxation by the eNOS activation pathway. Acupuncture stimulation caused temporary exchange nerve dominance and vasodilation. Bradykinin activates eNOS, resulting in vasorelaxation.

concentration of Ca^{2+}, which activates Cam and vasorelaxation by activating eNOS [171-173].

4.3. **Vasorelaxation by the iNOS activation pathway.** Figure 11 shows the flowchart of vasorelaxation by the iNOS activation pathway. At 30 min. to 1 hr. after tissue damage due to acupuncture/moxibustion stimulation, vascular permeability increases, plasma components infiltrate out of the blood vessels via gaps in the vessels and exude into the tissues [47, 174, 175], and leukocytes gather at the damaged site. Among the leukocytes, neutrophils generate prostaglandins [176]. In addition, monocytes exit from the blood vessels and become macrophages. Upon LPS and IFN stimulation, macrophages act on toll-like receptors on the cell surface to generate cytokines, such as IL-1β, IL-6, and TNF-α, and induce iNOS [66, 177-179]. SP and CGRP released by axon reflexes also secrete proinflammatory cytokines by activating monocytes [180].

Cytokines activate iNOS [67, 181-184], which then generates NO from L-arginine, resulting in vasorelaxation. Unlike nNOS and eNOS, iNOS does not require new Ca^{2+} because CaM is already bound [3, 56, 185].

The amount of NO produced by iNOS is considerably larger than those produced by nNOS and eNOS [7, 67, 74, 183]. However, iNOS is closely linked to the pathophysiology of inflammatory diseases and septic shock [6, 7, 76, 186].

5. **Discussion.** By collating the results of numerous studies in various fields, which cannot be performed by reading individual papers, the causal effects of the entire system from acupuncture/moxibustion stimulation to vasorelaxation could be inferred. In this

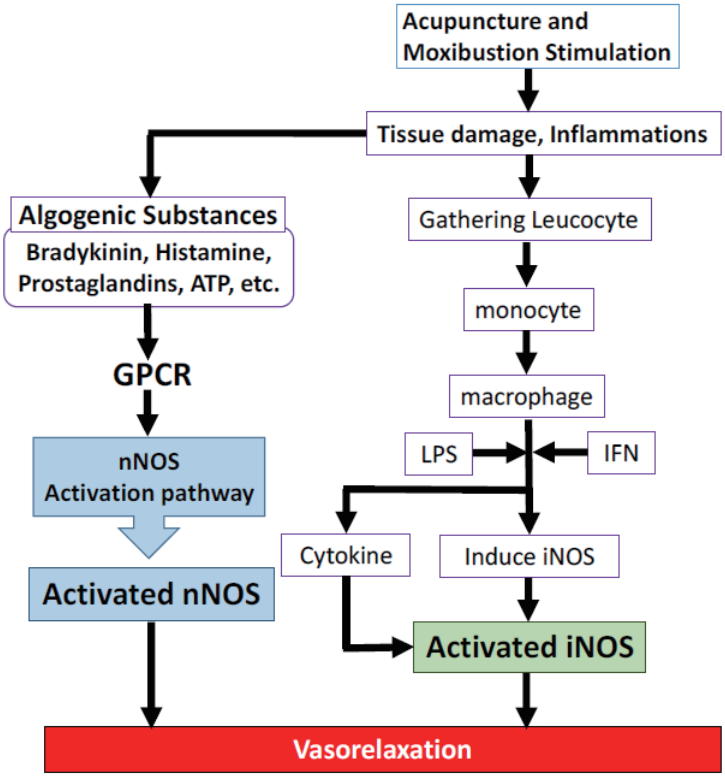


FIGURE 11. Vasorelaxation by the iNOS activation pathway. After tissue damage, leukocytes gather at the damaged site and monocytes become macrophages, which activate iNOS, leading to vasorelaxation.

review, we discuss the following models of vasorelaxation by acupuncture/moxibustion stimulation.

1) Nociceptive and thermal stimuli affect three types of NOS individually, namely, nNOS, eNOS, or iNOS, and the effects appear with time differences, leading to vasorelaxation and vasodilation [3]. It can be thought that nNOS, eNOS, and iNOS produce the NO in this order.
2) The effects of mechanical stimulation (pressure stimulation) causes nNOS and eNOS to induce vasorelaxation and vasodilation.

In short, acupuncture/moxibustion stimulation synthesizes L-citrulline (L-Cit) and NO from L-arginine (L-Arg) via three pathways, nNOS, eNOS, and iNOS activation pathways. Produced NO diffuses into the smooth muscle, vasorelaxation occurs. When NO is generated, endothelial cells exert an anti-arteriosclerotic effect by regulating the vascular tone, inhibiting platelet aggregation (anti-atherosclerosis), preventing leukocyte adhesion, and producing paracrine factors that limit VSM proliferation [19-22].

Therefore, the generation of NO limits the proliferation of VSM, and the vasorelaxation of the smooth muscle greatly affects atherosclerosis, Mönckeberg's arteriosclerosis, and fine arteriosclerosis. If arteriosclerosis can be prevented, many diseases, such as angina, myocardial infarction, and cerebral infarction, can be inhibited. In addition, arteriosclerosis prevention contributes to the prolonged delay of blood vessel aging.

This model is expected to lead to further research in various fields, including medicine, pharmacology, acupuncture/moxibustion, PT, OT, and other medical fields, and to the prevention of atherosclerosis. Researchers can explain the mechanism by which blood

vessels can be relaxed simply by applying pressure such as acupressure and massage, which are not noxious stimuli.

In the future, studies on acupuncture/moxibustion that are aimed at the extension of vasorelaxation and acupuncture/moxibustion effects are expected to progress. However, the continuous production of large amounts of NO by iNOS causes direct tissue toxicity, which leads to septic shock and hemorrhagic shock [7, 76].

TABLE 3. Summary of the features of nNOS, eNOS, and iNOS

	nNOS	**eNOS**	**iNOS**
Trigger stimuli for NO generation	• Pressure, thermal, chemical stimulation, and others • Increased intracellular Ca^{2+}	Vascular endothelial shear stress	• Increased intracellular Ca^{2+} • Cytokines
Stimulus	Acupuncture/ moxibustion stimulation	• Increased blood flow • Sympathetic dominance [45, 55, 169]	Stimulation from macrophages by leukocyte migration [7, 8, 50]
NOS expression	Nerve tissue (central and peripheral), lungs, kidneys, and others [7]	Vascular endothelial cells, bone marrow cells, platelets, and others [185]	• Immune system • Cardiovascular system • Lungs and others [5]
NOS expression in the skin [6]	Epidermal and pigment cells	Fibroblasts and vascular endothelial cells	Epidermal cells, Langerhans cells, fibroblasts, vascular endothelial cells, and macrophages
Existence of NOS	Postsynaptic density (PSD) cytoplasm [100, 132]	Localized in a special site called caveolae on the cell membrane [62, 63].	Cytoplasmic soluble, active as a homodimer [65]
NOS activity regulation	Calcium/calmodulin [3, 172]	Calcium/calmodulin [2, 172]	Induction of enzyme expression by cytokines Calcium-independent [54, 173-175]
Location of NO generation	Cytoplasm	Vascular endothelial cells Cytoplasm	Cytoplasm
NO generation mechanism	Produced as a byproduct of the conversion of L-arginine to L-citrulline by nNOS	Produced as a byproduct of the conversion of L-arginine to L-citrulline by eNOS	Produced as a byproduct of the conversion of L-arginine to L-citrulline by iNOS
Transfer of NO [55, 115, 118]	Diffusion	Diffusion	Diffusion

A noteworthy finding from the three path model is that the signal flow that originated from external stimulus is not only a straightforward path but also a complicated feedback path and circulation. The circulation and repeated stimulation may cause LTP.

As one of the preliminary experiments to verify this model of vasorelaxation by acupuncture stimulation, we examined the vasorelaxation of the hand finger before and after acupuncture with ring-headed thumbtack needles in 48 people by employing finger plethysmography. As a result, vasorelaxation was observed in most people. Interestingly even when ring-headed thumbtack needles were applied on the foot, vasorelaxation in the hand finger was also observed. From the results, we can infer that this response is an upper spinal reflex. The results of this experiment will be submitted for publication soon.

Table 3 summarizes the characteristics of the three NOSs from the references reviewed in this paper. Most of them have been described earlier.

6. **Conclusions.** We surveyed and correlated the existing literature in various academic fields. We, therefore, proposed a model of the mechanism of vasorelaxation by acupuncture/moxibustion stimulation. The results showed that the stimulation led to vasorelaxation via three pathways, nNOS based on neural information, eNOS based on vascular wall shear stress, and iNOS based on multiple cytokines produced by macrophages. The transmission of acupuncture/moxibustion stimulation includes a complicated feedback path and circulation, which are expected to lead to the long-term effect of vasorelaxation. We would like to continue experiments to validate this model.

Acknowledgments. We would like to express our deepest gratitude to the reviewers of IJICIC for their advice in compiling this paper. We would also like to acknowledge Professor Keiichi Horio and Mr. Kodai Kitagawa of the Graduate School of Kyushu Institute of Technology for their good suggestion and discussion.

REFERENCES

[1] World Health Organization, *Global Health Estimates 2016 Summary Tables*, 2016.
[2] W. K. Alderton, C. E. Cooper and R. G. Knowles, Nitric oxide synthases: Structure, function and inhibition, *The Biochemical Journal*, vol.357, no.3, pp.593-615, 2001.
[3] P. J. Andrew and B. Mayer, Enzymatic function of nitric oxide synthases, *Cardiovascular Research*, vol.43, no.3, pp.521-531, 1999.
[4] W. Xu, L. Z. Liu, M. Loizidou, M. Ahmed and I. G. Charles, The role of nitric oxide in cancer, *Cell Research*, vol.12, pp.311-320, 2002.
[5] K. Chen, R. N. Pittman and A. S. Popel, Nitric oxide in the vasculature: Where does it come from and where does it go?, *A Quantitative Perspective*, vol.10, no.7, pp.1185-1198, 2008.
[6] C. Villanuevaa and C. Giulivi, Subcellular and cellular locations of nitric oxide synthase isoforms as determinants of health and disease, *Free Radical Biology and Medicine*, vol.49, no.3, pp.307-316, 2010.
[7] U. Förstermann and W. C. Sessa, Nitric oxide synthases: Regulation and function, *European Heart Journal*, vol.33, no.7, pp.829-837, 2012.
[8] A. Mittal and R. Kakkar, Nitric oxide synthases and their inhibitors: A review, *Letters in Drug Design & Discovery*, vol.17, no.3, pp.228-252, 2020.
[9] M. Sandberg, T. Lundeberg, L.-G. Lindberg and B. Gerdle, Effects of acupuncture on skin and muscle blood flow in healthy subjects, *European Journal of Applied Physiology*, vol.90, nos.1-2, pp.114-119, 2003.
[10] K. Makiko, T. Katsumi, T. Yasukoet et al., Microcirculatory responses to acupuncture stimulation and phototherapy, *Anesthesia & Analgesia*, vol.108, no.2, pp.635-640, 2009.
[11] T. Kikuchi, M. Seto, S. Yamaguchi et al., Effect of electro-acupuncture stimulation on blood flow in trapezius muscle, *Kampo Medicine*, vol.61, no.6, pp.834-839, 2010.
[12] Q. Xu, J. Yang, L. Wang et al., Use of laser speckle contrast imaging to reveal changes in temperature and blood perfusion in the skin of healthy subjects after administration of heated moxa sticks and daiwenjiu ointment, *Journal of Traditional Chinese Medicine*, vol.34, no.4, pp.455-459, 2014.

[13] F. Kamali, H. M. A. Nematollahi, S. Heidari, E. Moosavi and M. Mohamadi, The effect of transcutaneous electrical nerve stimulation of sympathetic ganglions and acupuncture points on distal blood flow, *Journal of Acupuncture and Meridian Studies*, vol.10, no.2, pp.120-124, 2017.
[14] S. Uchida, H. Taniguchi, Y. Ito et al., Blood pressure-independent increase in the cortical cerebral blood flow induced by manual acupuncture of the auricular region in rats, *Journal of Physiological Science*, vol.69, pp.165-170, 2019.
[15] S. Lim, WHO standard acupuncture point locations, *Evidence Based Complementary Alternative Medicine*, vol.7, no.2, pp.167-168, 2010.
[16] World Health Organization, *Viewpoint on Acupuncture*, Geneva, Switzerland, 1979.
[17] K. Asako et al., New finding on fascia-traditional target of acupuncture, *Japanese Acupuncture and Moxibustion*, vol.15, no.1, pp.1-8, 2019.
[18] Y. Okami, M. Mukaida, H. Koga, R. Kawata, N. Suetake and E. Uchino, Objective evaluation of the degree of arteriosclerosis of retinal blood vessel and its change with age, *ICIC Express Letters*, vol.14, no.3, pp.235-241, 2020.
[19] M. W. Radomski, R. M. Palmer and S. Moncada, The anti-aggregating properties of vascular endothelium: Interactions between prostacyclin and nitric oxide, *British Journal of Pharmacology*, vol.92, no.3, pp.639-646, 1987.
[20] M. W. Radomski, R. M. J. Palmer and S. Moncada, The role of nitric oxide and cGMP in platelet adhesion to vascular endothelium, *Biochemical and Biophysical Research Communications*, vol.148, no.3, pp.1482-1489, 1987.
[21] A. M. Lefer and X. L. Ma, Decreased basal nitric oxide release in hypercholesterolemia increases neutrophil adherence to rabbit coronary artery endothelium, *Arteriosclerosis and Thrombosis*, vol.13, no.6, pp.771-776, 1993.
[22] U. Förstermann, Oxidative stress in vascular disease: Causes, defense mechanisms and potential therapies, *Nature Clinical Practice Cardiovascular Medicine*, vol.5, no.6, pp.338-349, 2008.
[23] Z. Pan, H. Yang and P. S. Reinach, Transient receptor potential (TRP) gene superfamily encoding cation channels, *Human Genomics*, vol.5, no.2, pp.108-116, 2011.
[24] L. Alonso, C. M. Kecskes, G. Jacobs et al., Muscling in on TRP channels in vascular smooth muscle cells and cardiomyocytes, *Cell Calcium*, vol.66, pp.48-61, 2017.
[25] A. Samanta, T. E. T. Hughes and V. Y. Moiseenkova-Bell, Transient receptor potential (TRP) channels, *Subcellular Biochemistry*, vol.87, pp.141-165, 2018.
[26] C. D. Benham, M. J. Gunthorpe and J. B. Davis, TRPV channels as temperature sensors, *Cell Calcium*, vol.33, nos.5-6, pp.479-487, 2003.
[27] J. Bockaert and J. P. Pin, Molecular tinkering of G protein-coupled receptors: An evolutionary success, *The EMBO Journal*, vol.18, no.7, pp.1723-1729, 1999.
[28] R. Fredriksson, M. C. Lagerström, L. G. Lundin and H. B. Schiöth, The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints, *Molecular Pharmacology*, vol.63, no.6, pp.1256-1272, 2003.
[29] C. D. Hanlon and D. J. Andrew, Outside-in signaling – A brief review of GPCR signaling with a focus on the Drosophila GPCR family, *Journal of Cell Science*, vol.128, no.19, pp.3533-3542, 2015.
[30] S. B. Gacasan, D. L. Baker and A. L. Parrill, G protein-coupled receptors: The evolution of structural insight, *AIMS Biophysics*, vol.4, no.3, pp.491-527, 2017.
[31] P. Geppetti, N. A. Veldhuis, T. M. Lieu and N. W. Bunnett, G protein-coupled receptors: Dynamic machines for signaling pain and itch, *Neuron*, vol.88, no.4, pp.635-649, 2015.
[32] D.-V. Predescu, S. M. Creţoiu, D. Creţoiu et al., G protein-coupled receptors (GPCRs)-mediated calcium signaling in ovarian cancer: Focus on GPCRs activated by neurotransmitters and inflammation-associated molecules, *International Journal of Molecular Sciences*, vol.20, no.22, p.5568, 2019.
[33] A. E. Dubin and A. Patapoutian, Nociceptors: The sensors of the pain pathway, *Journal of Clinical Investigation*, vol.120, no.11, pp.3760-3772, 2010.
[34] K. Messlinger, Was ist ein Nozizeptor? [What is a nociceptor?], *Anaesthesist*, vol.46, no.2, pp.142-145, 1997.
[35] S. N. Lawson, X. Fang and L. Djouhri, Nociceptor subtypes and their incidence in rat lumbar dorsal root ganglia (DRGs): Focussing on C-polymodal nociceptors, Aβ-nociceptors, moderate pressure receptors and their receptive field depths, *Current Opinion in Physiology*, vol.11, pp.125-146, 2019.
[36] V. B. Morhenn, N. S. Waleh, J. N. Mansbridge et al., Evidence for an NMDA receptor subunit in human keratinocytes and rat cardiocytes, *European Journal of Pharmacology: Molecular Pharmacology*, vol.268, no.3, pp.409-414, 1994.

[37] S. M. Carlton, G. L. Hargett and R. E. Coggeshall, Localization and activation of glutamate receptors in unmyelinated axons of rat glabrous skin, *Neuroscience Letters*, vol.197, no.1, pp.25-28, 1995.

[38] J. A. McRoberts, S. V. Coutinho, J. C. G. Marvizón et al., Role of peripheral N-methyl-D-aspartate (NMDA) receptors in visceral nociception in rats, *Gastroenterology*, vol.120, no.7, pp.1737-1748, 2001.

[39] S. M. Carlton, G. L. Hargett and R. E. Coggeshall, Localization of metabotropic glutamate receptors 2/3 on primary afferent axons in the rat, *Neuroscience*, vol.105, no.4, pp.957-969, 2001.

[40] A. D. Hogan-Cann and C. M. Anderson, Physiological roles of non-neuronal NMDA receptors, *Trends in Pharmacological Science*, vol.37, no.9, pp.750-767, 2016.

[41] D. D. Limbrick Jr, S. Sombati and R. J. DeLorenzo, Calcium influx constitutes the ionic basis for the maintenance of glutamate-induced extended neuronal depolarization associated with hippocampal neuronal death, *Cell Calcium*, vol.33, no.2, pp.69-81, 2003.

[42] C. Lüscher and R. C. Malenka, NMDA receptor-dependent long-term potentiation and long-term depression (LTP/LTD), *Cold Spring Harbor Perspectives in Biology*, vol.4, no.6, pp.1-15, 2012.

[43] J. K. Myung, A. W. Dunah, T. W. Yu and M. Sheng, Differential roles of NR2A- and NR2B-containing NMDA receptors in Ras-ERK signaling and AMPA receptor trafficking, *Neuron*, vol.46, no.5, pp.745-760, 2005.

[44] M. L. Blanke and A. M. J. Van Dongen, Activation mechanisms of the NMDA receptor, in *Biology of the NMDA Receptor, Chapter 13*, pp.283-312, CRC Press, 2009.

[45] P. F. Davies, Hemodynamic shear stress and the endothelium in cardiovascular pathophysiology, *Nature Clinical Practice Cardiovascular Medicine*, vol.6, no.1, pp.16-26, 2009.

[46] D.-L. Ren, A.-A. Sun, Y.-J. Li, M. Chen, S.-C. Ge and B. Hu, Exogenous melatonin inhibits neutrophil migration through suppression of ERK activation, *Journal of Endocrinology*, vol.227, no.1, pp.49-60, 2015.

[47] L. Pase, J. E. Layton, C. Wittmann et al., Neutrophil-delivered myeloperoxidase dampens the hydrogen peroxide burst after tissue wounding in zebrafish, *Current Biology*, vol.22, no.19, pp.1818-1824, 2012.

[48] P. Benly, Role of histamine in acute inflammation, *Journal of Pharmacological Science & Research*, vol.7, no.6, pp.373-376, 2015.

[49] B. Engelhardt and H. Wolburg, Mini review: Transendothelial migration of leukocytes: Through the front door or around the side of the house?, *European Journal of Immunology*, vol.34, no.11, pp.2955-2963, 2004.

[50] Y. Nonnenmacher and K. Hiller, Biochemistry of proinflammatory macrophage activation, *Cellular and Molecular Life Sciences*, vol.75, no.12, pp.2093-2109, 2018.

[51] H. K. Surks, cGMP-dependent protein kinase I and smooth muscle relaxation a tale of two isoforms, *Circulation Research*, vol.101, no.11, pp.1078-1080, 2007.

[52] K. S. Madhusoodanan and F. Murad, NO-cGMP signaling and regenerative medicine involving stem cells, *Neurocheical Research*, vol.32, nos.4-5, pp.681-694, 2007.

[53] A. Friebe and D. Koesling, Regulation of nitric oxide-sensitive guanylyl cyclase, *Circulation Research*, vol.93, no.2, pp.96-105, 2003.

[54] E. J. Tsai and D. A. Kass, Cyclic GMP signaling in cardiovascular pathophysiology and therapeutics, *Pharmacology & Therapeutics*, vol.122, no.3, pp.216-238, 2009.

[55] F. V. Brozovich, C. J. Nicholson, C. V. Degen, Y. Z. Gao, M. Aggarwal and K. G. Morgan, Mechanisms of vascular smooth muscle contraction and the basis for pharmacologic treatment of smooth muscle disorders, *Pharmacological Reviews*, vol.68, no.2, pp.476-532, 2016.

[56] H. J. Cho, Q. W. Xie, J. Calaycay et al., Calmodulln is a subunit of nitric oxide synthase from macrophages, *Journal of Experimental Medicine*, vol.176, no.2, pp.599-604, 1992.

[57] R. E. Coggeshall and S. M. Carlton, Ultrastructural analysis of NMDA, AMPA, and kainate receptors on unmyelinated and myelinated axons in the periphery, *The Journal of Comparative Neurology*, vol.391, no.1, pp.78-86, 1998.

[58] J. R. Vane, J. A. Mitchell and I. Appleton, Inducible isoforms of cyclooxygenase and nitric-oxide synthase in inflammation, *Proceedings of the National Academy of Sciences of the United States of America*, vol.91, no.6, pp.2046-2050, 1994.

[59] R. M. Rapoport and F. Murad, Agonist-induced endothelium-dependent relaxation in rat thoracic aorta may be mediated through cGMP, *Circulation Research*, vol.52, no.3, pp.352-357, 1983.

[60] L. J. Ignarro, R. G. Harbison, K. S. Wood and P. J. Kadowitz, Activation of purified soluble guanylate cyclase by endothelium-derived relaxing factor from intrapulmonary artery and vein: Stimulation by acetylcholine, bradykinin and arachidonic acid, *Journal of Pharmacology and Experimental Therapeutics*, vol.237, no.3, pp.893-900, 1986.

[61] S. H. Francis, J. L. Busch, J. D. Corbin and D. Sibley, cGMP-dependent protein kinases and cGMP phosphodiesterases in nitric oxide and cGMP action, *Pharmacological Reviews*, vol.62, no.3, pp.525-563, 2010.

[62] O. Feron, L. Belhassen, L. Kobzik, T. W. Smith, R. A. Kelly and T. Michel, Endothelial nitric oxide synthase targeting to caveolae. Specific interactions with caveolin isoforms in cardiac myocytes and endothelial cells, *Journal of Biological Chemistry*, vol.271, no.37, pp.22810-22814, 1996.

[63] P. W. Shaul, Endothelial nitric oxide synthase, caveolae and the development of atherosclerosis, *Journal of Physiology*, vol.15, no.547(Pt 1), pp.21-33, 2003.

[64] J. MacMicking, Q. W. Xie and C. Nathan, Nitric oxide and macrophage function, *Annual Reviews Immunology*, vol.15, pp.323-350, 1997.

[65] A. H. Sprague and R. A. Khalil, Inflammatory cytokines in vascular dysfunction and vascular disease, *Biochemical Pharmacology*, vol.78, no.6, pp.539-552, 2009.

[66] Y. I. Kim, S. W. Park, I. J. Kang, M. K. Shin and M. H. Lee, Activin suppresses LPS-induced Toll-like receptor, cytokine and inducible nitric oxide synthase expression in normal human melanocytes by inhibiting NF-κB and MAPK pathway activation, *International Journal of Molecular Medicine*, vol.36, no.4, pp.1165-1172, 2015.

[67] U. Förstermann and H. Kleinert, Nitric oxide synthase: Expression and expressional control of the three isoforms, *Naunyn-Schmiedeberg's Archives of Pharmacology*, vol.352, no.4, pp.351-364, 1995.

[68] R. C. Jin and J. Loscalzo, Vascular nitric oxide: Formation and function, *Journal of Blood Medicine*, vol.1, pp.147-162, 2010.

[69] I. Suzuki, K. Sakuraba, T. Horiike et al., A combination of oral L-citrulline and L-arginine improved 10-min full-power cycling test performance in male collegiate soccer players: A randomized crossover trial, *European Journal of Applied Physiology*, vol.119, pp.1075-1084, 2019.

[70] L. A. Blatter and W. G. Wier, Nitric oxide decreases $[Ca^{2+}]_i$ in vascular smooth muscle by inhibition of the calcium current, *Cell Calcium*, vol.15, no.2, pp.122-131, 1994.

[71] B. M. List, B. Klösch, C. Völker et al., Characterization of bovine endothelial nitric oxide synthase as a homodimer with down-regulated uncoupled NADPH oxidase activity: Tetrahydrobiopterin binding kinetics and role of haem in dimerization, *Biochemical Journal*, vol.323, no.1, pp.159-165, 1997.

[72] A. Presta, J. Liu, W. C. Sessa and D. J. Stuehr, Substrate binding and calmodulin binding to endothelial nitric oxide synthase co-regulate its enzymatic activity, *Nitric Oxide*, vol.1, no.1, pp.74-87, 1997.

[73] H. M. Abu-Soud and D. J. Stuehr, Nitric oxide synthases reveal a role for calmodulin in controlling electron transfer, *Proceedings of the National Academy of Sciences of the United States of America*, vol.90, no.22, pp.10769-10772, 1993.

[74] Y. Kobayashi, The regulatory role of nitric oxide in proinflammatory cytokine expression during the induction and resolution of inflammation, *Journal of Leukocyte Biology*, vol.88, no.6, pp.1157-1162, 2010.

[75] M. Tsutsui, H. Shimokawa, Y. Otsuji et al., Nitric oxide synthases and cardiovascular diseases, *Circulation Journal*, vol.73, no.6, pp.986-993, 2009.

[76] M. A. Titheradge, Nitric oxide in septic shock, *Biochimica et Biophysica Acta*, vol.1411, nos.2-3, pp.437-455, 1999.

[77] D. O. Schairer, J. S. Chouake, J. D. Nosanchuk and A. J. Friedman, The potential of nitric oxide releasing therapies as antimicrobial agents, *Virulence*, vol.3, no.3, pp.271-279, 2012.

[78] S. E. Kim, B. Coste, A. Chadha, B. Cook and A. Patapoutian, The role of Drosophila Piezo in mechanical nociception, *Nature*, vol.483, no.7388, pp.209-212, 2012.

[79] B. Coste, B. Xiao, J. S. Santos et al., Piezo proteins are pore-forming subunits of mechanically activated channels, *Nature*, vol.483, no.7388, pp.176-181, 2012.

[80] A. D. Pietra, N. Mikhailov and R. Giniatullin, The emerging role of mechanosensitive piezo channels in migraine pain, *International Journal of Molecular Science*, vol.21, no.3, p.696, 2020.

[81] B. Coste, J. Mathur, M. Schmidt et al., Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels, *Science*, vol.330, no.6000, pp.55-60, 2010.

[82] S. N. Bagriantsev, E. O. Gracheva and P. G. Gallagher, Piezo proteins: Regulators of mechanosensation and other cellular processes, *Journal of Biological Chemistry*, vol.289, no.46, pp.31673-31681, 2014.
[83] M. Tominaga and M. J. Caterina, Thermosensation and pain, *Journal of Neurobiology*, vol.61, no.1, pp.3-12, 2004.
[84] S. R. Garrison and C. L. Stucky, The dynamic TRPA1 channel: A suitable pharmacological pain target?, *Current Pharmaceutical Biotechnology*, vol.12, no.10, pp.1689-1697, 2011.
[85] F. Bezanilla, The action potential: From voltage-gated conductances to molecular structures, *Biological Research*, vol.39, no.3, pp.425-435, 2006.
[86] M. Kole, S. Ilschner, B. Kampa et al., Action potential generation requires a high sodium channel density in the axon initial segment, *Nature Neuroscience*, vol.11, no.2, pp.178-186, 2008.
[87] H. Liu, H. Wang, M. Sheng, L. Y. Jan, Y. N. Jan and A. I. Basbaum, Evidence for presynaptic N-methyl-D-aspartate autoreceptors in the spinal cord dorsal horn, *Proceedings of the National Academy of Sciences of the United States of America*, vol.91, no.18, pp.8383-8387, 1994.
[88] N. B. Lawand, T. McNearney and K. N. Westlund, Amino acid release into the knee joint: Key role in nociception and inflammation, *Pain*, vol.86, nos.1-2, pp.69-74, 2000.
[89] V. A. Carozzi, A. Canta, N. Oggioni et al., Expression and distribution of 'high affinity' glutamate transporters GLT1, GLAST, EAAC1 and of GCPII in the rat peripheral nervous system, *Journal of Anatomy*, vol.213, no.5, pp.539-546, 2008.
[90] K. E. Miller, E. M. Hoffman, M. Sutharshan and R. Schechter, Glutamate pharmacology and metabolism in peripheral primary afferents: Physiological and pathophysiological mechanisms, *Pharmacological Therapy*, vol.130, no.3, pp.283-309, 2011.
[91] R. Bardoni, Role of presynaptic glutamate receptors in pain transmission at the spinal cord level, *Current Neuropharmacology*, vol.11, no.5, pp.477-483, 2013.
[92] J. W. Little, T. Doyle and D. Salvemini, Reactive nitroxidative species and nociceptive processing: Determining the roles for nitric oxide, superoxide, and peroxynitrite in pain, *Amino Acids*, vol.42, no.1, pp.75-94, 2012.
[93] B. M. J. Olthof, S. E. Gartside and A. Rees, Puncta of neuronal nitric oxide synthase (nNOS) mediate NMDA receptor signaling in the auditory midbrain, *Journal of Neuroscience*, vol.39, no.5, pp.876-887, 2019.
[94] P. W. Mantyh, Neurobiology of substance P and the NK1 receptor, *Journal of Clinical Psychiatry*, vol.63, no.11, pp.6-10, 2002.
[95] M. Gautam, P. Prasoon, R. Kumar, K. H. Reeta, S. Kaler and S. B. Ray, Role of neurokinin type 1 receptor in nociception at the periphery and the spinal level in the rat, *Spinal Cord*, vol.54, no.3, pp.172-182, 2016.
[96] T. Nakazawa, S. Komai, T. Tezuka et al., Characterization of Fyn-mediated tyrosine phosphorylation sites on GluRε2 (NR2B) subunit of the N-methyl-D-aspartate receptor, *Journal of Biological Chemistry*, vol.276, no.1, pp.693-699, 2001.
[97] W. Guo, S. Zou, Y. Guan et al., Tyrosine phosphorylation of the NR2B subunit of the NMDA receptor in the spinal cord during the development and maintenance of inflammatory hyperalgesia, *Journal of Neuroscience*, vol.22, no.14, pp.6208-6217, 2002.
[98] T. Abe, S. Matsumura, T. Katano et al., Fyn kinase-mediated phosphorylation of NMDA receptor NR2B subunit at Tyr1472 is essential for maintenance of neuropathic pain, *European Journal of Neuroscience*, vol.22, no.6, pp.1445-1454, 2005.
[99] R. Sattler, Z. Xiong, W. Y. Lu, M. Hafner, J. F. MacDonald and M. Tymianski, Specific coupling of NMDA receptor activation to nitric oxide neurotoxicity by PSD-95 protein, *Science*, vol.284, no.5421, pp.1845-1848, 1999.
[100] K. S. Christopherson, B. J. Hillier, W. A. Lim and D. S. Bredt, PSD-95 assembles a ternary complex with the N-methyl-D-aspartic acid receptor and a bivalent neuronal NO synthase PDZ domain, *Journal of Biological Chemistry*, vol.274, no.39, pp.27467-27473, 1999.
[101] S. E. Tan, R. J. Wenthold and T. R. Soderling, Phosphorylation of AMPA-type glutamate receptors by calcium/calmodulin-dependent protein kinase II and protein kinase C in cultured hippocampal neurons, *Journal of Neuroscience*, vol.14, no.3(Pt 1), pp.1123-1129, 1994.
[102] J. Z. Yan, Z. Xu, S. Q. Ren et al., Protein kinase C promotes N-methyl-D-aspartate (NMDA) receptor trafficking by indirectly triggering calcium/calmodulin-dependent protein kinase II (CaMKII) autophosphorylation, *Journal of Biological Chemistry*, vol.286, no.28, pp.25187-25200, 2011.
[103] A. Barria and R. Malinow, NMDA receptor subunit composition controls synaptic plasticity by regulating binding to CaMKII, *Neuron*, vol.48, no.2, pp.289-301, 2005.

[104] R. S. Petralia, R. A. Al-Hallaq and R. J. Wenthold, Trafficking and targeting of NMDA receptors, in *Biology of the NMDA Receptor, Chapter 8*, pp.149-200, CRC Press, 2008.
[105] J. Y. Lan, V. A. Skeberdis, T. Jover et al., Protein kinase C modulates NMDA receptor trafficking and gating, *Nature Neuroscience*, vol.4, no.4, pp.382-390, 2001.
[106] J. Lisman, R. Yasuda and S. Raghavachari, Mechanisms of CaMKII action in long-term potentiation, *Journal of Biological Chemistry*, vol.286, no.28, pp.25187-25200, 2011.
[107] R. C. Carroll and R. S. Zukin, NMDA-receptor trafficking and targeting: Implications for synaptic transmission and plasticity, *Trends in Neuroscience*, vol.25, no.11, pp.571-577, 2002.
[108] J. C. Hee, H. H. Yan, L. F. Lau and R. L. Huganir, Regulation of the NMDA receptor complex and trafficking by activity-dependent phosphorylation of the NR2B subunit PDZ ligand, *Journal of Neuroscience*, vol.24, no.45, pp.10248-10259, 2004.
[109] I. Pérez-Otaño and M. D. Ehlers, Learning from NMDA receptor trafficking: Clues to the development and maturation of glutamatergic synapses, *Neurosignals*, vol.13, no.4, pp.175-189, 2004.
[110] L. Groc, M. Heine, L. Cognet et al., Differential activity-dependent regulation of the lateral mobilities of AMPA and NMDA receptors, *Nature Neuroscience*, vol.7, no.7, pp.695-696, 2004.
[111] H. B. Kwon and P. E. Castillo, Long-term potentiation selectively expressed by NMDA receptors at hippocampal mossy fiber synapses, *Neuron*, vol.57, no.1, pp.108-120, 2008.
[112] D. L. Hunt and P. E. Castillo, Synaptic plasticity of NMDA receptors: Mechanisms and functional implications, *Current Opinions in Neurobiology*, vol.22, no.3, pp.496-508, 2012.
[113] T. V. P. Bliss and S. F. Cooke, Long-term potentiation and long-term depression: A clinical perspective, *Clinics*, vol.66, no.1, pp.3-17, 2011.
[114] T. V. P. Bliss and T. Lømo, Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path, *The Journal of Physiology*, vol.232, no.2, pp.331-356, 1973.
[115] P. G. Jorens, P. A. Vermeire and A. G. Herman, L-arginine-dependent nitric oxide synthase: A new metabolic pathway in the lung and airways, *European Respiratory Journal*, vol.6, pp.258-266, 1993.
[116] R. Davydov, K. J. Labby, S. E. Chobot et al., Enzymatic and cryoreduction EPR studies of the hydroxylation of methylated N(ω)-hydroxy-L-arginine analogues by nitric oxide synthase from geobacillus stearothermophilus, *Biochemistry*, vol.53, no.41, pp.6511-6519, 2014.
[117] J. H. Shin and D. J. Linden, An NMDA receptor/nitric oxide cascade is involved in cerebellar LTD but is not localized to the parallel fiber terminal, *Journal of Neurophysiology*, vol.94, no.6, pp.4281-4289, 2005.
[118] O. V. Evgenov, P. Pacher, P. M. Schmidt, G. Haskó, H. H. H. W. Schmidt and J. P. Stasch, NO-independent stimulators and activators of soluble guanylate cyclase: Discovery and therapeutic potential, *Nature Reviews Drug Discovery*, vol.5, no.9, pp.755-768, 2006.
[119] J. Schlossmann and M. Desch, IRAG and novel PKG targeting in the cardiovascular system, *American Journal of Physiology Heart and Circulatory Physiology*, vol.301, no.3, pp.H672-H682, 2011.
[120] S. Iyengar, M. H. Ossipov and K. W. Johnson, The role of calcitonin gene-related peptide in peripheral and central pain mechanisms including migraine, *Pain*, vol.158, no.4, pp.543-559, 2017.
[121] I. J. Lever, E. J. Bradbury, J. R. Cunningham et al., Brain-derived neurotrophic factor is released in the dorsal horn by distinctive patterns of afferent fiber stimulation, *Journal of Neuroscience*, vol.21, pp.4469-4477, 2001.
[122] S. D. Brain, T. J. Williams, J. R. Tippins, H. R. Morris and I. MacIntyre, Calcitonin gene-related peptide is a potent vasodilator, *Nature*, vol.313, no.5997, pp.54-56, 1985.
[123] N. Charkoudian, Skin blood flow in adult human thermoregulation: How it works, when it does not, and why, *Mayo Clinical Proceedings*, vol.78, no.5, pp.603-612, 2003.
[124] K. Tasaka, Recent advances in the research on histamine release, *Folia Pharmacologica Japonica*, vol.98, no.3, pp.197-207, 1991.
[125] S. D. Brain and T. J. Williams, Interactions between the tachykinins and calcitonin gene-related peptide lead to the modulation of oedema formation and blood flow in rat skin, *British Journal of Pharmacology*, vol.97, no.1, pp.77-82, 1989.
[126] F. A. Pinho-Ribeiro, W. A. Verri Jr and I. M. Chiu, Nociceptor sensory neuron-immune interactions in pain and inflammation, *Trends in Immunology*, vol.38, no.1, pp.5-19, 2017.
[127] M. Tominaga, M. Wada and M. Masu, Potentiation of capsaicin receptor activity by metabotropic ATP receptors as a possible mechanism for ATP-evoked pain and hyperalgesia, *Proceedings of the National Academy of Sciences of the United States of America*, vol.98, no.12, pp.6951-6956, 2001.

[128] M. Numazaki, T. Tominaga, H. Toyooka and M. Tominaga, Direct phosphorylation of capsaicin receptor VR1 by protein kinase Cε and identification of two target serine residues, *Journal of Biological Chemistry*, vol.277, no.16, pp.13375-13378, 2002.
[129] T. Sugiura, M. Tominaga, H. Katsuya and K. Mizumura, Bradykinin lowers the threshold temperature for heat activation of vanilloid receptor 1, *Journal of Neurophysiology*, vol.88, no.1, pp.544-548, 2002.
[130] V. A. Aroniadou and A. Keller, Mechanisms of LTP induction in rat motor cortex in vitro, *Cerebral Cortex*, vol.5, no.4, pp.353-362, 1995.
[131] K. P. S. J. Murphy, G. P. Reid, D. R. Trentham and T. V. P. Bliss, Activation of NMDA receptors is necessary for the induction of associative long-term potentiation in area CA1 of the rat hippocampal slice, *Journal of Physiology*, vol.504, no.2, pp.379-385, 1997.
[132] J. V. Esplugues, NO as a signalling molecule in the nervous system, vol.135, no.5, pp.1079-1095, 2002.
[133] A. Latremoliere and C. J. Woolf, Central sensitization: A generator of pain hypersensitivity by central neural plasticity, *Journal of Pain*, vol.10, no.9, pp.895-926, 2009.
[134] J. M. McHugh and W. B. McHugh, Pain: Neuroanatomy, chemical mediators, and clinical implications, *AACN Clinical Issues*, vol.11, no.2, pp.168-178, 2000.
[135] B. L. Kidd and L. A. Urban, Mechanisms of inflammatory pain, *British Journal of Anaesthesia*, vol.87, no.1, pp.3-11, 2001.
[136] L. A. Abdulkhaleq, M. A. Assi, R. Abdullah, M. Zamri-Saad, Y. H. Taufiq-Yap and M. N. M. Hezmee, The crucial roles of inflammatory mediators in inflammation: A review, *Veterinary World*, vol.11, no.5, pp.627-635, 2018.
[137] L. Sun and R. D. Ye, Role of G protein-coupled receptors in inflammation, *Acta Pharmacology*, vol.33, pp.342-350, 2012.
[138] T. Renné, K. Schuh and W. Müller-Esterl, Local bradykinin formation is controlled by glycosaminoglycans, *Journal of Immunology*, vol.175, no.5, pp.3377-3385, 2005.
[139] C. Golias, A. Charalabopoulos, D. Stagikas, K. Charalabopoulos and A. Batistatou, The kinin system-bradykinin: Biological effects and clinical implications. Multiple role of the kinin system-bradykinin, *Hippokratia*, vol.11, no.3, pp.124-128, 2007.
[140] J. Bryant and Z. Shariat-Madar, Human plasma kallikrein-kinin system: Physiological and biochemical parameters, *Cardiovascular & Hematological Agents in Medicinal Chemistry*, vol.7, no.3, pp.234-250, 2009.
[141] A. C. Brooks, C. J. Whelan and W. M. Purcell, Reactive oxygen species generation and histamine release by activated mast cells: Modulation by nitric oxide synthase inhibition, *British Journal of Pharmacology*, vol.128, no.3, pp.585-590, 1999.
[142] F. Borriello, R. Iannone and G. Marone, Histamine release from mast cells and basophils, *Handbook of Experimental Pharmacology*, vol.241, pp.121-139, 2017.
[143] D. Fujisawa, J. Kashiwakura, H. Kita et al., Expression of mas-related gene X2 on mast cells is upregulated in the skin of patients with severe chronic urticarial, *The Journal of Allergy and Clinical Immunology*, vol.134, no.3, pp.622-633, 2014.
[144] W. D. Dachman, G. Bedarida, T. F. Blaschke and B. B. Hoffman, Histamine-induced venodilation in human beings involves both H1 and H2 receptor subtypes, *The Journal of Allergy and Clinical Immunology*, vol.93, no.3, pp.606-614, 1994.
[145] J. M. Brown, T. M. Wilson and D. D. Metcalfe, The mast cell and allergic diseases: Role in pathogenesis and implications for therapy, *Clinical and Experimental Allergy*, vol.38, no.1, pp.4-18, 2008.
[146] K. Amin, The role of mast cells in allergic inflammation, *Respiratory Medicine*, vol.106, no.1, pp.9-14, 2012.
[147] E. Ricciotti and G. A. Fitzgerald, Prostaglandins and inflammation, *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol.31, no.5, pp.986-1000, 2011.
[148] A. Scott, K. M. Khan, C. R. Roberts, J. L. Cook and V. Duronio, What do we mean by the term "inflammation"? A contemporary basic science update for sports medicine, *British Journal of Sports Medicine*, vol.38, no.3, pp.372-380, 2004.
[149] T. A. Butterfield, T. M. Best and M. A. Merrick, The dual roles of neutrophils and macrophages in inflammation: A critical balance between tissue damage and repair, *Journal of Athletic Training*, vol.41, no.4, pp.457-465, 2006.
[150] L. P. Feigen, Actions of prostaglandins in peripheral vascular beds, *Federation Proceedings*, vol.40, no.7, pp.1987-1990, 1981.

[151] C. Dees, A. Akhmetshina, P. Zerr et al., Platelet-derived serotonin links vascular disease and tissue fibrosis, *Journal of Experimental Medicine*, vol.208, no.5, pp.961-972, 2011.
[152] P. M. Vanhoutte, Serotonin and the vascular wall, *International Journal of Cardiology*, vol.14, no.2, pp.189-203, 1987.
[153] R. Wisastra and F. J. Dekker, Inflammation, cancer and oxidative lipoxygenase activity are intimately linked, *Cancers (Basel)*, vol.6, no.3, pp.1500-1521, 2014.
[154] C. C. Chan and A. Ford-Hutchinson, Effects of synthetic leukotrienes on local blood flow and vascular permeability in porcine skin, *Journal of Investigative Dermatology*, vol.84, no.2, pp.154-157, 1985.
[155] F. Amaya, Y. Izumi, M. Matsuda and M. Sasaki, Tissue injury and related mediators of pain exacerbation, *Current Neuropharmacology*, vol.11, no.6, pp.592-597, 2013.
[156] H. Ishizaka and L. Kuo, Acidosis-induced coronary arteriolar dilation is mediated by ATP-sensitive potassium channels in vascular smooth muscle, *Circulation Research*, vol.78, no.1, pp.50-57, 1996.
[157] E. E. M. Van Ginneken, P. Meijer, N. Verkaik, P. Smits and G. A. Rongen, ATP-induced vasodilation in human skeletal muscle, *British Journal of Pharmacology*, vol.141, no.5, pp.842-850, 2004.
[158] Y. Hellsten, M. Nyberg, L. G. Jensen and S. P. Mortensen, Vasodilator interactions in skeletal muscle blood flow regulation, *The Journal of Physiology*, vol.590, no.24, pp.6297-6305, 2012.
[159] Y. Ren, J. Garvin and O. A. Carretero, Mechanism involved in bradykinin-induced efferent arteriole dilation, *Kidney International*, vol.62, no.2, pp.544-549, 2002.
[160] H. Y. Kwan, P. C. Leung, Y. Huang and X. Yao, Depletion of intracellular Ca^{2+} stores sensitizes the flow-induced Ca^{2+} influx in rat endothelial cells, *Circulation Research*, vol.92, no.3, pp.286-292, 2003.
[161] Y. Kawai, M. Kaidoh, Y. Yokoyama and T. Ohhashi, Pivotal roles of shear stress in the microenvironmental changes that occur within sentinel lymph nodes, *Cancer Science*, vol.103, no.7, pp.1245-1252, 2012.
[162] K. Sriram, J. G. Laughlin, P. Rangamani and D. M. Tartakovsky, Shear-induced nitric oxide production by endothelial cells, *Biophysical Journal*, vol.111, no.1, pp.208-221, 2016.
[163] H. Sakakibara, S. Iwase, T. Mano et al., Skin sympathetic activity in the tibial nerve triggered by vibration applied to the hand, *International Archives of Occupational and Environmental Health*, vol.62, no.6, pp.455-458, 1990.
[164] A. Sato, Y. Sato, A. Suzuki and S. Uchida, Neural mechanisms of the reflex inhibition and excitation of gastric motility elicited by acupuncture-like stimulation in anesthetized rats, *Neuroscience Research*, vol.18, no.1, pp.53-62, 1993.
[165] S. Knardahl, M. Elam, B. Olausson and B. G. Wallin, Sympathetic nerve activity after acupuncture in humans, *Pain*, vol.75, no.1, pp.19-25, 1998.
[166] H. Tada, M. Fujita, M. Harris et al., Neural mechanism of acupuncture-induced gastric relaxations in rats, *Digestive Disease Science*, vol.48, pp.59-68, 2003.
[167] K. Kimura, K. Masuda and I. Wakayama, Changes in skin blood flow and skin sympathetic nerve activity in response to manual acupuncture stimulation in humans, *American Journal of Chinese Medicine*, vol.34, no.2, pp.189-196, 2006.
[168] Y. Q. Li, B. Zhu, P. J. Rong, H. Ben and Y. H. Li, Neural mechanism of acupuncture-modulated gastric motility, *World Journal of Gastroenterology*, vol.13, no.5, pp.709-716, 2007.
[169] G. M. Buga, M. E. Gold, J. M. Fukuto and L. J. Ignarro, Shear stress-induced release of nitric oxide from endothelial cells grown on beads, *Hypertension*, vol.17, no.2, pp.187-193, 1991.
[170] R. Gordan, J. K. Gwathmey and L.-H. Xie, Autonomic and endocrine control of cardiovascular function, *World Journal of Cardiology*, vol.7, no.4, pp.204-214, 2015.
[171] M. B. Harris, H. Ju, V. J. Venema et al., Reciprocal phosphorylation and regulation of endothelial nitric-oxide synthase in response to bradykinin stimulation, *Journal of Biological Chemistry*, vol.276, pp.16587-16591, 2001.
[172] J. L. Lowry, V. Brovkovych, Y. Zhang and R. A. Skidgel, Endothelial nitric-oxide synthase activation generates an inducible nitric-oxide synthase-like output of nitric oxide in inflamed endothelium, *Journal of Biological Chemistry*, vol.288, no.6, pp.4174-4193, 2013.
[173] Y. Zhao, P. M. Vanhoutte and S. W. S. Leung, Vascular nitric oxide: Beyond eNOS, *Journal of Pharmacological Sciences*, vol.129, no.2, pp.83-94, 2015.

[174] D. A. Steeber, M. A. Campbell, A. Basit, K. Ley and T. F. Tedder, Optimal selectin-mediated rolling of leukocytes during inflammation in vivo requires intercellular adhesion molecule-1 expression, *Proceedings of the National Academy of Sciences of the United States of America*, vol.95, no.13, pp.7562-7567, 1998.

[175] S. K. Yoo, T. W. Starnes, Q. Deng and A. Huttenlocher, Lyn is a redox sensor that mediates leukocyte wound attraction in vivo, *Nature*, vol.480, no.7375, pp.109-112, 2011.

[176] H. L. Wright, R. J. Moots, R. C. Bucknall and S. W. Edwards, Neutrophil function in inflammation and inflammatory diseases, *Rheumatology (Oxford)*, vol.49, no.9, pp.1618-1631, 2010.

[177] L. Chen, H. Deng, H. Cui et al., Inflammatory responses and inflammation-associated diseases in organs, *Oncotarget*, vol.9, no.6, pp.7204-7218, 2018.

[178] Q. Wang, X. Zhou, L. Yang et al., Gentiopicroside (GENT) protects against sepsis induced by lipopolysaccharide (LPS) through the NF-κB signaling pathway, *Annals of Translational Medicine*, vol.7, no.23, p.731, 2019.

[179] Q. Chaochao, G. Lou, Y. Yang et al., Macrophage inflammatory protein-2 in high mobility group box 1 secretion of macrophage cells exposed to lipopolysaccharide, *Cellular Physiology and Biochemistry*, vol.42, no.3, pp.913-928, 2017.

[180] M. Schäffer, T. Beiter, H. D. Becker and T. K. Hunt, Neuropeptides: Mediators of inflammation and tissue repair?, *Archives of Surgery*, vol.133, no.10, pp.1107-1116, 1998.

[181] D. Beasley and M. Eldridge, Interleukin-1 beta and tumor necrosis factor-alpha synergistically induce NO synthase in rat vascular smooth muscle cells, *The American Journal of Physiology*, vol.266, no.4(Pt 2), pp.R1197-R1203, 1994.

[182] K. A. Kirkebøen and O. A. Strand, The role of nitric oxide in sepsis – An overview, *Acta Anaesthesiologica Scandinavica*, vol.43, no.3, pp.275-288, 1999.

[183] F. Aktan, iNOS-mediated nitric oxide production and its regulation, *Life Science*, vol.75, no.6, pp.639-653, 2004.

[184] P. Sagoo, G. Chan, D. F. P. Larkin and A. J. T. George, Inflammatory cytokines induce apoptosis of corneal endothelium through nitric oxide, *Investigative Ophthalmology & Visual Science*, vol.45, no.11, pp.3964-3973, 2004.

[185] A. J. Hobbs, A. Higgs and S. Moncada, Inhibition of nitric oxide synthase as a potential therapeutic target, *Annual Review of Pharmacology and Toxicology*, vol.39, pp.191-220, 1999.

[186] M. S. Carraway, C. A. Piantadosi, C. P. Jenkinson and Y.-C. T. Huang, Differential expression of arginase and iNOS in the lung in sepsis, *Experimental Lung Research*, vol.24, no.3, pp.253-268, 1998.

Author Biography

Tetsuya Yoneda graduated from the Department of Applied Chemistry, Faculty of Engineering, Kagoshima University in 1981, and started working for a major company. He has been working in the field of semiconductor electroplating technology for 25 years. He was working abroad in management for a total of 22 years.

He passed the national examination for acupuncture and moxibustion therapists in 2019. In the same year, he enrolled in the Ph.D. course of Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology. He is currently a graduate student, an acupuncturist and a consultant in human resource development. His research interest lies in the scientific aspects of acupuncture and moxibustion treatment.

Takeshi Yamakawa is a Professor Emeritus of Kyushu Institute of Technology (KIT) and the Founding Director of Fuzzy Logic Systems Institute (FLSI), both in Japan. He is also acting as a practitioner in acupuncture and moxibustion therapy.

He received the B. Eng. degree in electronics engineering in 1969 from Kyushu Institute Technology, Tobata and the M. Eng. degree in electronics engineering in 1971 from Tohoku University, both in Japan. He received the Ph.D. degree for his studies on electrochemical devices in 1974 from Tohoku University, Japan.

His main research interest lies in hardware implementation of fuzzy systems, fuzzy neural networks, and chaotic systems, and also application of dielectrophoresis to clinical laboratory automation. His current interests include scientific clarification of oriental medicine. He holds 11 patents in U.S.A., 4 patents in Europe, 1 patent in Australia and 1 patent in Taiwan, and he has also applied for more than 120 patents in Japan. Prof. Yamakawa is a fellow of IEEE, International Fuzzy Systems Association (IFSA) and Japan Society of Fuzzy Theory and Systems (SOFT). He received IEEE 2008 Fuzzy Systems Pioneer Award. He is acting as a member of editorial board and a regional editor of 10 international professional journals. He contributed more than 80 international conferences as a member or the chairman of organizing/programming committee. He was used to organize the International Conference on Fuzzy Logic, Neural Nets and Soft Computing (so called IIZUKA Conference) every two years in Iizuka, Japan.

Prof. Yamakawa plays Karate (Japanese traditional martial arts) and possesses a black belt (5th Dan). And he likes swimming, a monocycle and horse riding as well. His interest also lies in Shakuhachi and Shamisen, which are Japanese traditional musical instruments.

Chikamune Wada received his B. Eng. degree in mechanical engineering from Osaka University, Japan, in 1990 and his Ph.D. degree in biomedical engineering from Hokkaido University, Japan, in 1996.

From 1996 to 2001, he was an Assistant Professor with the Sensory Information Laboratory, at Hokkaido University. In 2001, he became an Associate Professor with Human-Function Substitution System Laboratory, Kyushu Institute of Technology. Since 2016, he has been a Professor with the Human-Function Substitution System Laboratory. His research interests include assistive technology, especially measuring human motion and informing disabled people of the necessary information to improve their QOL. He is a member of the IEEE, the Institute of Electronics, Information and Communication Engineers (IEICE) and Japan Human Factors and Ergonomics Society. And, he was one of the chief editors for the journal of the IEICE.