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Integrins and Nitric Oxide in the Regulation of Glia Cells: Potential Roles in Pathological Pain

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Abstract

Glial cells form physical connections with neurons and vascular endothelial cells in the brain, thereby constructing the elaborate networks of the tripartite synapse and the neurovascular unit, respectively. In addition to supporting neurons, glial cells modify synaptic plasticity and vascular tone, and in this way play an important role in maintaining brain homeostasis. Upon activation, glial cells produce nitric oxide, a gaseous mediator that diffuses into neighboring cells, wherein it elicits signaling pathways critical for synaptic plasticity and vascular tone. Because nitric oxide is short-lived and can travel only a short distance, glial cells must migrate to and position themselves near the target cells with which glial nitric oxide primarily interacts. Integrins, an essential family of cell adhesion molecules, facilitate effective glial migration and adhesion in the brain during developmental and normal adult stages. Aberrant regulation of nitric oxide and integrins in glial cells is thought to compromise cognitive brain function, and thereby lead to various pathologies. This review focuses on the important pathologic roles that nitric oxide and integrins play in glia and glia-related cells, focusing on their potential involvement in chronic pain syndrome.

Keywords: Astrocytes; Microglia; Neuropathic Pain; Nitric Oxide; Integrin; Inflammation

Introduction

Pain can be both physiological and pathological [1-3]. The physiological pain experienced upon injury serves as a necessary warning sign to the organism (e.g., animal) in order to avoid further trauma to the tissue. Once the pain is felt, an animal can alter its behavior to avoid the noxious stimuli. Wound pain is usually exacerbated with movement. Thus, pain leads to guarding behaviors, facilitating the healing process by stabilizing the wound. This pain is physiological pain and is known as noxious pain. It typically disappears after the wound heals a common human experience.

The physiologic mechanism by which pain sensation is elicited has been demonstrated both at the cellular and molecular levels. Noxious stimuli activate transducer ion channels located in the free nerve endings of the Aδ and C nociceptors, which induce generator potential (receptor potential) and, subsequently, action potential [3,4]. The action potential of a primary afferent fiber is transmitted to the dorsal horn, where the primary neuron synapses to the secondary neuron. A neurotransmitter, such as glutamate and/or substance P (SP), is released from the presynaptic ending. Glutamate binds and activates the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) in the postsynaptic membrane, causing a subsequent influx of Na^+ into the postsynaptic neuron and producing excitatory postsynaptic potential. This ultimately opens the voltage-activated Na^+ channel (NaV), which induces action potential in the postsynaptic neuron. Thus, the initial noxious stimuli are now transmitted via the spinal cord to the brain, where the pain is perceived. The Aδ nociceptor releases glutamate and produces a sharp initial, localized, and rapid sensation of pain. The C nociceptor then releases both glutamate and SP, producing a dull, slower, un-localized secondary feeling of pain. SP binds and activates the neurokinin-1 (NK-1) receptor in the postsynaptic membrane of the secondary neuron.

In contrast to physiologic pain, which is a transient and self-limiting phenomenon, pathological pain persists even in the absence of the initial noxious stimuli. The etiology of chronic pain syndrome or pathological pain includes, but is not limited to, nerve damage, postherpetic neuralgia, diabetes, and AIDS, among many others [1-4]. The clinical manifestations of pathological pain can include the following: pain in response to normally innocuous stimuli (allodynia), increasing magnitudes of pain due to noxious stimuli (hyperalgesia), or spontaneous pain, which persists long after the initial injury is cured, all of which are classified as unnecessary pain.

At the center of the pathological pain lies the aberrant transmission at the synapse, which involves the functional and organic alteration of pre- and post-synaptic neuronal membranes [3]. Glial cells were once thought to play merely a housekeeping role with regards to neurons. In fact, glial cells provide structural and metabolic support for neurons, which they accomplish via the uptake of amino acid neurotransmitters such as the glutamate released from presynaptic neurons. Glial cells have been the focus of increasing scientific inquiry as a key player both in synaptic plasticity and in pathological pain. For example, impairment in the capacity of glial cells to uptake glutamate could cause an increase in synaptic glutamate levels [5]. The activation of glia and proinflammatory cytokine release has been implicated in states of chronic non-ordinary pain. Of the mediators produced by - and acting on - activated glial cells, the gaseous mediator nitric oxide (NO) has been shown to act as an important player in pathological pain. Furthermore, in order for activated glial cells to migrate to sites of injury where they can release NO, integrins, that prominent family of cell adhesion molecules, become of paramount importance. Here, we review the roles played by nitric oxide and integrins in the regulation of glial functions in both the physiology and pathophysiology of chronic pain.

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Role of Nitric Oxide in the Regulation of Glial Function

Nitric oxide in the vascular and central nervous systems

NO is well known as an important endothelium-derived vasodilator in the vascular system, where it reacts with guanylyl cyclase to form cGMP, which activates cGMP-dependent protein kinase. This leads to the activation of such proteins as the Ca^{2+}-sensitive potassium channel, thereby reducing the cytoplasmic Ca^{2+} concentration of vascular smooth muscle cells [6]. Inhaled NO is currently used as a selective pulmonary vasodilator in clinical settings such as persistent neonatal pulmonary hypertension, acute lung injury, and the perioperative care associated with heart surgery [7].

The production of NO in the nervous system was first described in the cerebellum, where the stimulation of NMDA (N-methyl-D-aspartate) receptors by glutamate was shown to produce a substance later identified as NO [8]. NO is produced in the postsynaptic neurons and glial cells during conditions of pathological pain [1,3,4,5]. Ca^{2+}, through the activated NMDA receptor, stimulates nNOS, thereby producing NO postsynaptically. NO is then diffused from the nerve fiber, allowing it to reach activated glia as well as presynaptic neurons [9,10] (Figure 1).

At physiologic levels, NO is protective and preserves cell function. It becomes cytotoxic, however, when produced at high levels or when combined under pro-oxidant conditions. In the latter setting, NO becomes a cytotoxic reactive nitrogen species (RNS) such as ONOO- or nitrogen (III) oxide [11]. NO reacts with oxygen, transitional metal ions, thiols, and superoxide [12], exerting its effects in a cyclic guanosine-3',5'-monophosphate (cGMP)-dependent and/or -independent manner. cGMP effector molecules include the cGMP-dependent protein kinases type-I and –II, cGMP-activated phosphodiesterases, and cGMP-gated ion channels [10]. Like phosphorylation, NO regulates protein functions post-translationally by S-nitrosylation,

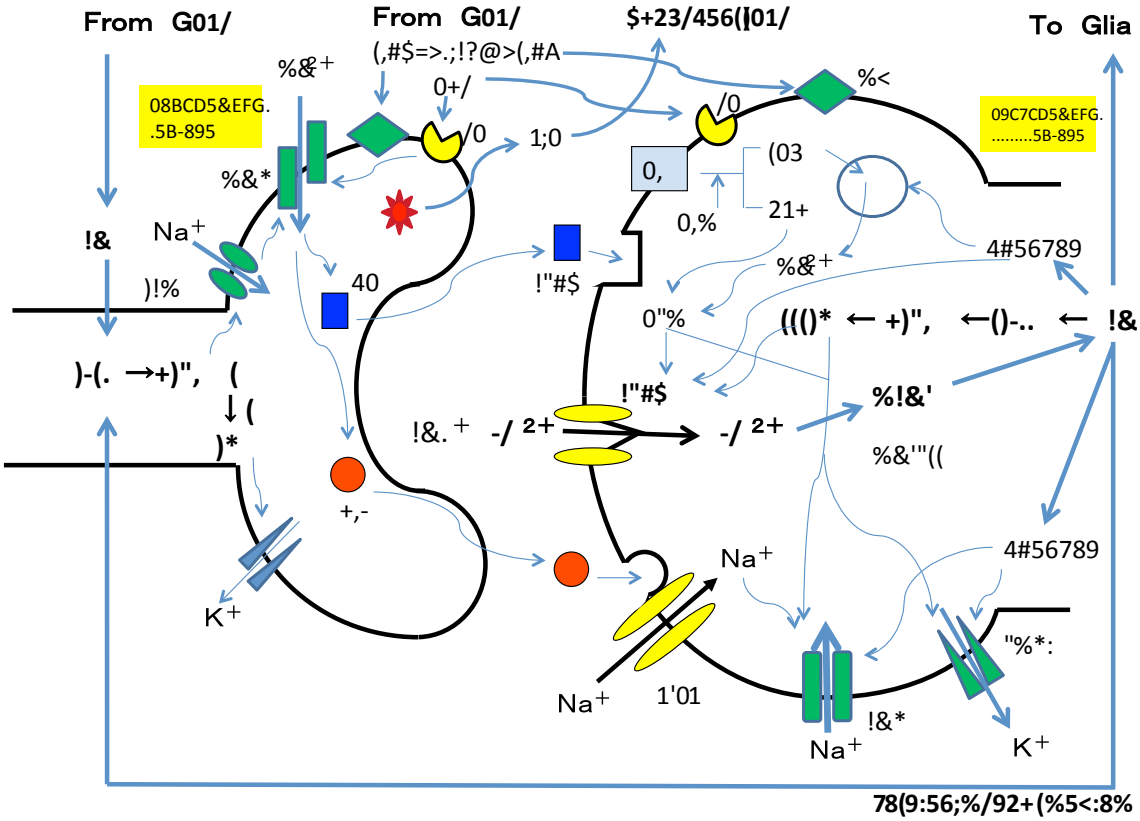

Figure 1: Postsynaptic NMDA-NOS-NO pathway enhances presynaptic glutamate release and postsynaptic transmission, and activates glia in persistent pain. In acute pain, glutamate, SP, and ATP are released from the presynaptic neuron. Glutamate activates AMPA receptor and induces generator potential, which opens NaV causing action potential. Once the acute noxious stimuli and inflammatory response has gone, the pain would disappear. When repetitive noxious stimulation continues, SP activates NK-1 receptor, causing PLC activation making IP3 and DAG from phospholipid. IP3 stimulates Ca2+ release from Ca2+ stored site. Increased Ca2+ and DAG activates C kinase which phosholylates NMDA receptor with subsequent Ca2+ and Na+ influx into the postsynaptic secondary neuron, followed by activation of nNOS, NO production, Guanylyl cyclase activation, cGMP formation, activation of GK, inducing the further activation of NMDA receptor. NO produced in the secondary neuron diffuses out of the neuron and reach the presynaptic neuron and neighboring glial cell. In the presynaptic neuron NO-induced GK activation closes K channel with its phosphorylation and opens HNC, causing depolarization with subsequent activation of Ca2+ channel. Increase in Ca2+ releases the glutamate and SP into the synaptic cleft causing vicious cycle.

AMPA,α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ATP, adenosine triphosphate; CaV, voltage-activated calcium channel; cGMP, cyclic guanosine-3',5'monophosphate; CaMK II, calcium calmodulin kinase II; DAG, diacylglycerol; EP, prostaglandin E receptor; GC, guanylyl cyclase; GK, cGMP dependent protein kinase; Glu, glutamate; SP, substance P; HNC, hyperpolarization-activated cyclic nucleotide-gated channels ; IP3, inositol triphosphate; NK-1, neurokinin-1 receptor; NaV, voltage-activated sodium channel; NMDA, N-methyl-D-aspartate; nNOS, neuronal nitric oxide synthase: NO, nitric oxide; PGE, prostaglandin E; PKC, protein kinase C; PLC, phospholipase C; S-nitro, S-nitrosylation; SKF, Src-family kinase;

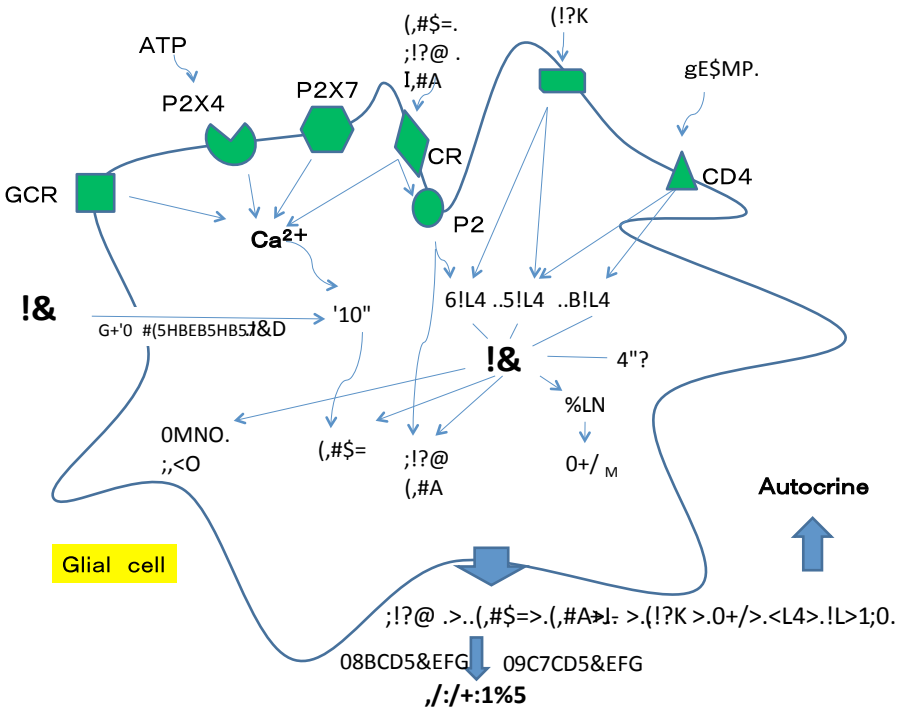

Figure 2: Activated glial cell produces neuroactive mediator and responds to neuroactive mediator with special references to NO. Glial cell is activated by inflammatory mediator, bacteria, virus, and nerve injury. Nerve injury produces ATP from presynaptic neuron and activates glial cell. NO comes from postsynaptic neuron in persistent pain state and activates glia through MAPK activation (Figure 1). Activated glia reduces uptake of glutamate, increasing synaptic glutamate concentration, intensifying pain. IL-1,IL-6, TNFα,INFγ,and gp120 induces NO production via NOS with subsequent increase in IL-1,IL-6, TNFα,INFγ, and PGE2, working on itself, neighboring glia and neurons with autocrine and paracrine manner.

ATP, adenosine triphosphate; COX, cyclooxygenase; cGMP, cyclic guanosine monophosphate; CR, cytokine receptor; GCR, G-protein coupled receptor; IL, interleukin; INF, interferon; MAPK, mitogen-activated protein kinase; NOS, nitric oxide synthase(inducible, neuronal, endothelial); NO, nitric oxide; PGE, prostaglandin E; P2X, ionotropic purinoceptor; P2, ionotropic purinoceptor(P2X) or G-protein-coupled pyrimidinergic receptor(P2Y); ROS, reactive oxygen species; TLR, Toll-like receptor; TNF, tumor necrosis factor;

which modifies protein functionality by altering the resident cysteine residues [13], (see Section D for protein S-nitrosylation and integrin functions).

NO in pathological pain

In conditions of pathological pain, altered states of synaptic transmission have been observed, including the following: an increase in neurotransmitter release from the primary afferent neurons [9,10]; activation of NMDA receptor in secondary neurons [14-18]; and activation of Glia cell [1-3,5,18-23]. All of these alterations favor intensification in synaptic transmission, thereby resulting in hyperalgesia and allodynia.

Once the NMDA receptor becomes activated, the vicious cycle of pathological pain is switched on. The cycle starts with the presynaptic release of glutamate and SP, leading to the activation of NMDA receptors, which induces a postsynaptic increase in Ca^{2+} concentration, as well as an increase in neuronal nitric oxide synthase (nNOS) activity. This finally results in the production of large quantities of NO, which is then diffused to the presynaptic fiber, where it induces glutamate release, thereby maintaining pain levels. Glia cells join in this vicious cycle by producing NO. Activation of any of these steps can result in the onset of this vicious cycle. Expression of the NR2B-bearing NMDA receptor is known to be upregulated under the following settings: in chronic nerve constriction injury, in chronic compression of the dorsal root ganglia [24,25], and in oxaliplatin-induced neuropathic pain, in which NOS inhibition reduces pain levels [17].

In states of pathological pain, NO is produced in dorsal horn secondary neurons and in glial cells, exerting its effects therein and then diffusing to presynaptic neurons [9]. The presynaptic cGMP formed by NO-guanylyl cyclase-1 facilitates glutamate release. The activation of hyperpolarization-activated cyclic nucleotide-gated channels (HCN), which directly bind cGMP or cyclic-3', 5' adenosine monophosphate (cAMP) and induce inward current (depolarization), might well be the cause of transmitter release [10]. NMDA receptor activation induces postsynaptic NO production, which further activates NMDA and AMPA receptors, in this case as a positive feedback mechanism, via the activation of cGMP-dependent protein kinases [4] , which ultimately intensifies the synaptic transmission [10,16]. The phosphorylation of ion channels for sodium and potassium regulates inward (depolarizing) current, which facilitates synaptic transmission [26], while S-Nitrosylation downregulates NMDA receptor activity. NO is capable of nitrosylate cysteine residues found in the NR1 and NR2 subunits of the NMDA receptor [13]. NO can also induce S-nitrosylation of NaV 1.5, potassium channel (KCNQ), as well as the Ca^{2+} channel (L-type, RyR) [13]. The effects of S-nitrosylation for each specific subtype must still be determined. For example, whereas NO activates the NMDA receptor via the cGMP pathway, NO suppresses the receptor via S-nitrosylation.

Glial cells and NO

Dorsal horn microglia and astrocytes are activated by subcutaneous inflammation, peripheral nerve injury, peritonitis (intraperitoneal bacterial infection), bone cancer, lumber spinal nerve constriction, spinal cord trauma, and immune activation within the spinal cord [20]. In one nerve injury model, microglia in the spinal cord was activated as shown by the increased expression of an ionotropic purinoceptor 4(P2X4) receptor and a p38 mitogen-activated protein kinase (MAPK) [2].

Functionally activated glia cells produce neuroactive substances including NO, the cytokines interleukin(IL)-1 and IL-6, tumor necrosis factor (TNF), reactive oxygen species (ROS), prostaglandins (PGs), excitatory amino acids (EAAs), interferon (INF)γ, and adenosine triphosphate (ATP) [20,21] (Figure 2). These substances have autocrine and paracrine effects, which exaggerate the action, in a feed-forward fashion, of the glia itself and/or neighboring neurons and glia in the spinal cord. Inflammatory mediators activate G-protein coupled receptors and activate ion channels via adenylate cyclase-induced cAMP-dependent protein kinase activation, most likely in neurons and glia cells [26]. Thus, glial cells not only release neurotransmitters, but also respond to neurotransmitters. In addition, toll-like receptor 4 (TLR-4) and P2X4 receptors are expressed on activated glial cells.

NO is an important mediator of hyperalgesia in chronic pain syndromes occurring at the spinal dorsal horn [1]. In human fetal astrocytes, IL-1β stimulated the expression of NOS, TNFα, and IL-6 in mRNA and protein levels, which decreases in the presence of the P2 pyrogenic nucleotide receptor antagonist, suggesting that IL-1β increases iNOS expression through P2 receptor activation [27]. The reverse also occurs, wherein HIV-gp120-induced NO increases the release of IL-1β, TNFα, IL-6 in spinal cord glia [23].

In astrocytes the activation of c-Jun N-terminal kinase (JNK), a member of the MAPK family, leads to the production of IL-1β [14]. Glutamate stimulates the NMDA receptor-nNOS pathway, producing NO in the postsynaptic neuron. NO diffuses out of the neuron, reaching astrocytes and activating JNK through a non-cGMP-dependent pathway. Astroglial JNK activation contributes to the positive feedback loop of pain exacerbation during the excitatory neuronal transmission. Interferon (IFN)γ has been implicated in the activation of glia responsible for neuropathic pain. IFNγ induced increased iNOS and nNOS mRNA expression in a mouse microglial-cultured cell line. The NO donor sodium nitroprusside (SNP) increased the mRNA expression of IL-6, Toll-like receptor (TLR) 4, and P2X4 receptors in the same glial cell line, suggesting that NO activates glia [21]. Nerve injury-induced Src-family kinase (SKFs) upregulation in glia is suppressed in mice lacking three NOS genes (eNOS, iNOS, nNOS) [21], suggesting that NO activates glial function in neuropathic pain. Among the three NOSs, nNOS may be important for spinal activation of microglia.

IL-β and TNFα induce PGE2 formation in astroglial cells, in part through the activation of cyclooxygenases, which occurs via the activation of the L-arginine-NO pathway [22]. NO and PGE2 block the ATP-induced migration of glia and retain microglia in the spinal cord [19], potentially explaining the increased numbers of glial cells observed in conditions pathological pain. Thus, glial cells are a crucial source of NO and PG in pathologic transmission of pain. PGE_2, coupled with the EP1 receptor, induces Ca^{2+} influx in a presynaptic manner, resulting in the release of glutamate, which activates the NMDA receptor. The opioid μ receptor is present in astroglial cells, its emergence coupled to the release of NO. In addition, morphine is known to increase glial fibrillary acidic protein levels, leading to the development of morphine tolerance [28]. Glial cells may be involved in opioid tolerance through the production of NO and PG [28], since nitric oxide inhibition reduces opioid tolerance [29].

NMDA-NOS pathway for LTP

Long-term potentiation (LTP) is a characteristic phenomenon in memory formation and learning, which involves the use-dependent facilitation of synaptic transmission. The onset of NMDA receptor activation initiates a sequence of key events in the postsynaptic neuron that leads to the formation of LTP [15]. Upon NMDA receptor activation, which leads to elevated levels of cytosolic Ca^{2+} in the postsynaptic neuron, several key Ca^{2+}-dependent pathways are activated, including such secondary messengers as kinases (MAPK, protein kinase A, protein kinase C, phosphatidyl inositol (PI) 3 kinase, Src), or NOS.

In hippocampal LTP, NO is produced postsynaptically by nNOS, which is a causative process since NOS inhibition reduces LTP [30]. In one study, LTP was reduced in mice lacking endothelial and neuronal nitric oxide synthase [31]. NO increases neurotransmitter release in the presynaptic terminal and enhances postsynaptic response in a cGMP-dependent manner since the guanylyl cyclase inhibitor reduces LTP [32]. Among guanylyl cyclases (GCs), GC-1 might be presynaptically localized in order to increase neurotransmitter release, while GC-2 would remain postsynaptically located [33]. GC-1 might play a role in fear memory, while GC-2 may do the same for anxiety in amygdala [34]. cGMP, in a postsynaptic manner, increases cGMP-dependent protein kinase II, which regulates AMPA receptor trafficking, thereby enhancing overall synaptic response [16] and increasing excitatory postsynaptic potential. The phosphorylation of NR2B in the NMDA receptor is involved in LTP in settings of persistent neuropathic pain [18].

Although LTP is necessary in hippocampal cells for memory and learning, its presence in the dorsal horn secondary neuron is problematic since it can result in unnecessary pain. Any available NMDA receptor inhibitors reduce LTP in both hippocampal cells and in the dorsal horn secondary neuron, which impairs memory and learning. For treatment purposes, a selective NMDA receptor antagonist that affects only the dorsal horn secondary neuron still awaits discovery, and is indeed under investigation. Because NO is produced by spinal glial cells in situations of pathological pain, specifically suppressing specifically only dorsal horn glial NO production might be of some therapeutic benefit.

Integrins in the Immune and Central Nervous Systems

The role of integrins in the pathogenesis of neuropathic pain is currently thought to be two-fold. First, the integrins on neutrophils and macrophages mediate the accumulation of these immune cells at sites of neuronal injury, thereby exacerbating inflammation, which is known to worsen pain [35,36]. Second, integrins on glia and neurons might play an active and direct role in modifying the specific signals involved in the pathogenesis of neuropathic pain. The inhibition of integrins has been shown to block the induction of hyperalgesia in neuropathic pain models [37,38]. Interestingly, intact alpha1, alpha3, and beta1 integrin subunit functionality are required in PGE_2-induced hyperalgesia, which involves protein kinase A signaling. In contrast, intact alpha5 and beta1 integrin subunit functionality are essential to epinephrine-induced hyperalgesia, which involves a combined signaling cascade consisting of protein kinase A, protein kinase C epsilon, and mitogen-activated protein kinase/extracellular signal-

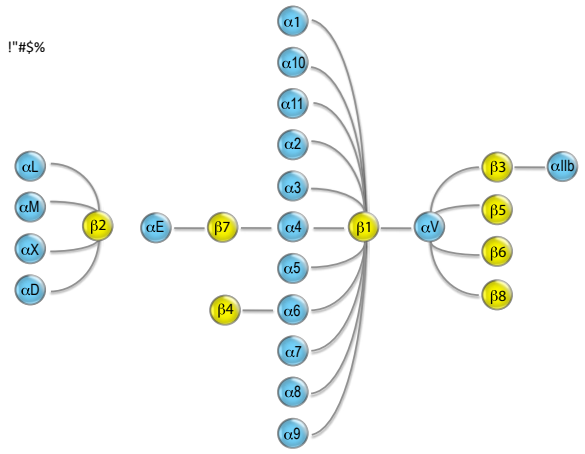

Figure 3: Integrin subunits. Eighteen alpha- and eight beta-subunits form 24 integrin heterodimers.

regulated kinase [39]. Furthermore, it has been found that beta1 and beta3 integrins colocalize with the mu opioid receptor in rat trigeminal ganglion neurons, wherein they regulate opiate receptor signaling [40]. This might constitute an alternative by which integrin signaling modifies pain. In the sections that follow, we describe the structure and functions, as well as the physiologic and pathologic roles played by integrins in the immune and central nervous systems.

Integrins and their roles in the immune system

Integrins are a family of cell adhesion molecules containing non-covalently-associated alpha and beta subunits [41-44]. To date, 18 different integrin alpha subunits and eight different beta subunits have been reported in vertebrates, forming at least 25 alpha/beta heterodimers (Figure 3). This perhaps reveals integrins to be the most structurally and functionally diverse family of cell adhesion molecules yet known. Integrins mediate cell-cell and cell-extracellular matrix interactions over a wide range of biological contexts. Not only do integrins support force-resistant stable firm adhesion, they are also involved in the dynamic adhesive interactions observed in cellular polarization and cell migration. Integrin-dependent physiological processes include tissue morphogenesis, inflammation, wound healing, and the regulation of cell growth and differentiation. Glial cells have been shown to express multiple beta1, beta2, beta3, and beta8 integrins [45].

Integrins are known for their unique ability to transmit bi-directional transmembrane signals [41]. Once intracellular signaling pathways are activated by other receptors (e.g., receptors coupled to G proteins or tyrosine kinases), the signals impinge on integrin cytoplasmic domains and enhance the activity of the extracellular headpiece for ligand binding via global conformational changes (inside-out signaling). Conversely, the binding of ligand to the extracellular part of the integrin initiates intracellular signaling via conformational changes to the cytoplasmic domains (outside-in signaling). Dynamic and reversible conversion of integrins between non-adhesive and adhesive states is made possible by such bi-directional signaling.

The adhesive and signaling activities of integrins are vital to many of the cell-cell and cell-extracellular matrix interactions involved in immune responses [46]. Integrins on hematologic cells play a critical role in their adhesive interactions with endothelial cells during migration to lymphoid organs and extravasation to sites of inflammation. The physiologic importance of integrins on hematologic cells is illustrated by two rare genetic disorders: leukocyte adhesion deficiency type I (LAD-I) and type III (LAD-III). LAD-I is caused by loss-of-function mutations in the beta2 integrin subunit that result in the absence, or severely reduced expression, of all beta2 integrin heterodimers on the cell surface of leukocytes [47,48]. LAD-I patients suffer from recurrent and often life-threatening bacterial infections and from impaired wound healing, since beta2 integrins are important for host defenses against microorganisms. Neutrophils from LAD-I patients showed a markedly reduced capacity to adhere to endothelial cells and to migrate to sites of inflammation. LAD-1 lymphocytes exhibited impaired function in antigen- and mitogen-induced proliferation, antibody-dependent killing, and T cell-dependent antibody production. LAD-III is caused by a genetic defect in kindlin-3, a cytoskeletal protein that activates integrins by binding to integrin b cytoplasmic tails. LAD-III patients manifest not only an increased susceptibility to bacterial infections, such as occurs as with LAD-I, but also to platelet dysfunction [49]. The latter can be observed in Glanzmann thrombasthenia, where one finds a lack of integrin alphaIIb/beta3 expression or functionality. Despite normal levels of integrin expression, these same LAD-III leukocytes failed to upregulate alphaL/beta2, alphaM/beta2, and alpha4/beta2 adhesiveness in response to chemokines and/or the other chemoattractants that activate GPCR signaling.

Structures of integrin heterodimers and integrin domains: Integrins are alpha/beta heterodimeric membrane proteins. A characteristic feature of integins lies in their complex multi-domain organization. Each integrin subunit is a type I transmembrane protein composed of a large extracellular segment, a single transmembrane segment, and a short cytoplasmic tail (except for the beta4 subunit, which contains a long cytoplasmic tail). The C-termini of the alpha and beta subunits associate with each other to form a globular ligand-binding headpiece. This headpiece is connected to the plasma membrane via the leg pieces (Figure 4A).

Half of the alpha subunits and all of the beta subunits contain a von Willebrand factor-type A domain of ~200 amino acids, known as an inserted (I) domain [44,50]. The I domains adopt a Rossmann fold that contains a metal ion-dependent adhesion site (MIDAS) located on the top, whereas its C- and N-terminal connections located on the distal bottom face (Figure 4; A and E) [44,51-53]. The ability of the I domain to bind ligand is regulated by conformational changes. The affinity of the I domain for its ligand is dramatically enhanced by a “piston-like” downward axial displacement of its C-terminal helix (arrow 1 in Figure 4E). The C-terminal downward shift is conformationally linked to the conversion of the MIDAS to the high-affinity configuration that tightly binds to ligand (arrow 2 in Figure 4E) [54-57]. Conversely, the binding of ligand to the MIDAS induces its high-affinity configuration, which has been linked to a downward axial displacement of the C-terminal helix [51,56,58].

The activation of other receptors - including TCR and chemokine receptors and probably several growth factor receptors - initiates an intracellular signaling cascade that eventually impinges upon the integrin cytoplasmic tails. Binding to the integrin cytoplasmic domains of signaling molecules (e.g., talin, kindlin) triggers a dissociation of the integrin cytoplasmic tails [59-61]. Separation of the alpha and beta subunit cytoplasmic tails has been linked to a separation of the transmembrane domains from the membrane-proximal segments of the extracellular domains, thereby inducing a switchblade-like opening that leads to an extended conformation [41,43]. This extension facilitates

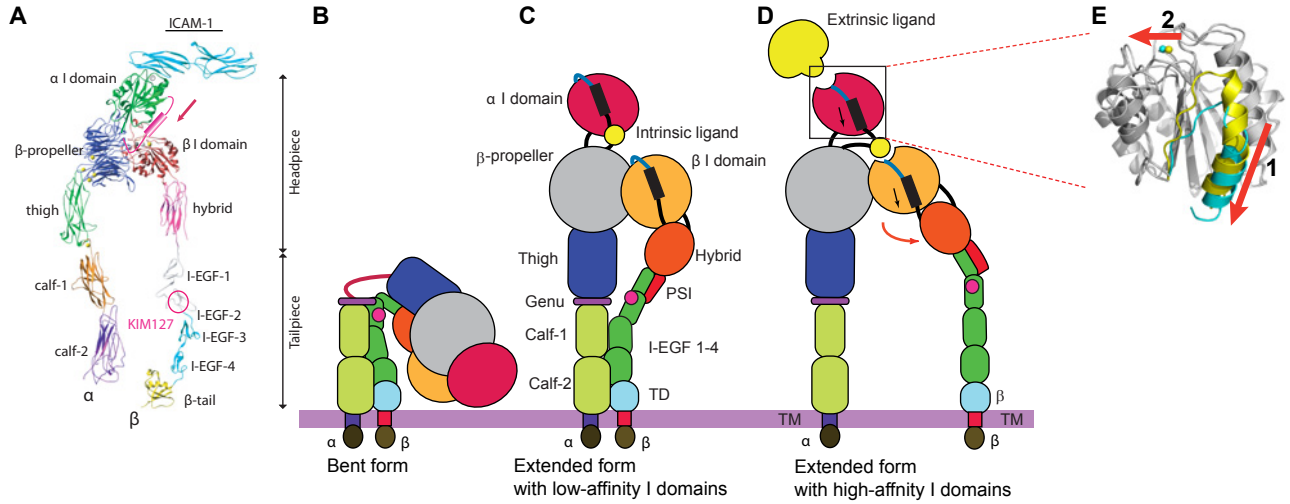

Figure 4: Integrin structures and domains and conformational changes. (A) Integrin extracellular segment model. (B-E) Global conformational changes between the bent (B), intermediate (C), and extended (D) conformations. Blow-ups (E) showing the structures of the high- and low-affinity conformations of the alpha I domain. A piston-like downward shift of the C-terminal helix (arrow 1) is allosterically linked to the conversion of the MIDAS to the high-affinity configuration (arrow 2). Superposition of the high- (blue) and closed low- (yellow) affinity I domain is shown. Regions undergoing significant conformational changes are colored, whereas regions not undergoing significant conformational changes are in gray.

the outward movement of the hybrid domain, which is coupled to the downward piston-like movement of the C-terminal helix (Figure 4; B-D). This downward shift of the beta I domain's C-terminal alpha helix triggers the conversion of the MIDAS into an open configuration, thereby binding to the C-terminal portion of the alpha I domain as an "internal" ligand [62]. This inter-domain interaction leads to a downward shift of the alpha I domain C-terminal helix, converting it to the high-affinity conformation, which binds to an "external" ligand.

Integrins in the central nervous system

Formation of the neuronal synapse: In the CNS, integrins are implicated in mediating both neuron-to-neuron, as well as glia-to-neuron, interactions [45,63]. Integrins expressed at the neuronal synapse have been shown to modulate synaptic plasticity [64]. An RGD peptide represents a consensus motif found in many (but not all) integrin ligands (e.g., collagen, laminin, fibronectin) and is used to block a subset of integrin-ligand interactions. Earlier investigations applied RGD peptides to hippocampal slices, which suggest that interfering with integrins compromised LTP [64,65]. A major breakthrough occurred when it was discovered that a fruit fly Drosophila mutant contained a short-term memory defect, now known as the locus of Volado (a Chilean word for 'forgetful'), which encodes an alpha integrin [66]. Integrins are thought not only to participate in strengthening the physical contact of neurons at the synapse, but also to have some involvement in the signaling that occurs at the interface. This idea has been further substantiated by a series of investigations using knockout mice that lack the expression of specific integrins [67-72].

Glial cells have physical contacts with pre- and post-synaptic neuronal cell membranes, thereby surrounding the neuronal synaptic gap to form a "tripartite synapse" [73,74]. At the tripartite synapse, glial cells communicate with neurons bidirectionally. Glial cells not only are affected by neurotransmitters secreted from neurons, but also impact on synaptic functions by secreting several molecules that modify neuronal activities [63,75]. A notable example is the amino acid D-serine that is produced exclusively in astrocytes [76]. D-serine binds to an agonist (glycine)-binding site of the NMDA receptor, thereby opening the Ca2+ channels. By secreting D-serine into the synapse, astrocytes contribute to long-term potentiation [77].

An array of cell adhesion molecules including integrins, cadherins, neurexins, and neuroligins have been shown to be important for the formation of neuron-neuron contact at the synapse [63]. Although cell adhesion molecules mediating glia-neuron interactions at the synapse remain less well studied, integrins are thought to be an important player in mediating glia-neuron interactions at the tripartite synapse [63]. Of note, a family of large multi-domain extracellular matrix proteins thrombospondins is a key molecule that is produced by astrocytes and deposited at the synapse. On the one hand, thrombospondins interact with the alpha2/delta1-1 subunit of the voltage-dependent Ca^{2+} channel on neurons, thereby transmitting a critical signal for synaptic development [78]. On the other hand, thrombospondisn interact with integrins, potentially supporting the adhesion of glial cells with neurons at the synapse. One investigation using embryonic retinal ganglion neurons has demonstrated that neurons are capable of forming, but not receiving, synapses unless physical contact with astrocytes had been established [79]. The integrin alphaVbeta3 on astrocytes has been shown to bind to a glycoprotein Thy-1 abundantly expressed on neurons [80]. Integrin binding to neuronal Thy-1 results in the activation of astrocytes. In turn, the astrocyte integrin induces clustering of neuronal Thy-1, thereby leading to the inhibition of neurite outgrowth and to the retraction of neuronal processes [81]. This bi-directional astrocyte-neuron communication via integrins might play an important role in the process of axon repair following neuronal damage. Another investigation using neurons isolated from the rat hippocampus has shown that the beta1 integrin on neurons mediates their contact with local astrocytes, thereby facilitating the transmission of signals that induce neuronal PKC activation, which globally enhances excitatory synaptogenesis [82].

Glial migration to sites of injury and regeneration: In response to a CNS injury, which leads to a release of inflammatory mediators such as ATP, cytokines and chemokines, glial cells - including both microglia

and astrocytes - become activated and migrate to injury sites [83]. This accumulation of glial cells at sites of injury is termed reactive gliosis. Beta 1 integrins have been shown to regulate astrocyte migration [84]. Beta1 integrins and matrix metalloprotease-2 (MMP-2) are co-localized at the leading edge of migrating astrocytes, where they cooperate to make their way through a mesh of extracellular matrix proteins including collagen, laminin, fibronectin, and thrombospondins. Beta 1 integrins have also been implicated in astrocyte migration to sites of spinal cord injury [85]. Injured neurons secrete the chemokine MCP-1, thereby attracting and activating astrocytes [86]. Astrocytes that migrated to, and were activated at, the dorsal root ganglions release IL-1 and TNF, which have been implicated in the pathogenesis of neuropathic pain [87,88].

Schwann cells represent a subset of glial cells at the peripheral nervous system. The migration of Schwann cells is important for tissue repair, specifically remyelination in injured demyelinated nerves. Schwann cell migration has been shown to depend on integrins and to be inhibited by the aggrecan produced by astrocytes [89]. In ensheathing and myelinating neuronal axons, Schwann cells form a laiminin-deposited organized basal lamina, in which beta1 and beta4 integrins are engaged. The Schwann cell-specific conditional knockout of laminin-binding beta1 integrins demonstrated that beta 1-null Schwann cells do not maintain normal processes around axons [90]. A subsequent investigation employing a Schwann cell-specific knockout of beta4 integrin has shown the essential role alpha6beta4 integrin plays in axonal regeneration and subsequent myelination [91].

Integrins and connexins in the neurovascular unit: Astrocytes make contact not only with neurons, but also with such brain blood vessel components as vascular endothelial cells and pericytes [92]. Throughout the brain vasculature, astrocytes, neurons, and endothelial cells are interconnected, and, thereby form a closely knitted coupling cellular network termed the neurovascular unit [93]. While astroglial integrins are thought to be important for stabilizing the endfeet attachment that enwraps blood vessels, another family of cell adhesion molecules known as connexins is enriched at the interface between astroglial and endothelial cells, where they form numerous gap junctions [94]. Gap junctions are built by two hemichannnels, called a connexon that contains six connexin proteins [95]. Gap junctions connect the cytoplasm of neighboring cells and allow the passages of intracellular mediators such as ions, amino acids, small metabolites, and second messengers. In the CNS, gap junctions provide the mechanism of cell-to-cell communication, and play an important role in maintaining normal and pathogenic condition [96]. Among 21 different connexins in human, connexin 43 and connexin 30 are the major connexins expressed in astrocytes [97]. Connexin 37, connexin 40 and connexin 43 are expressed by smooth muscle cells and endothelial cells in vascular wall [98]. Astroglial cells send signals to vascular endothelial cells via communication links extending through these gap junctions, and in this way regulate vascular tones (vasodilation or vasoconstriction).

In addition to the potential role astroglial integrins may play in stabilizing endfeet contact with vascular endothelial cells in a developed neurovascular unit [99], these integrins also play a pivotal role during the neurovascular unit's developmental stages [93]. Conditional knockout of either alphaV [100] or beta8 integrin [101] in the CNS has been shown to compromise proper development of the neurovascular unit, resulting in premature death due to multiple brain pathologies including cerebral hemorrhage. Interestingly, mice deficient in TGF-beta have been shown to develop CNS pathologies that are nearly identical to those in mice lacking alphaV or beta8 integrin [102]. This premature development of the neurovascular unit observed in TGF-beta, as well as in alphaV and beta integrin, knockout mice can be explained by the fact that alphaVbeta8 and alphaVbeta6 integrins have a unique ability to activate the TGF-beta signaling pathway [103]. TGF-beta is stored in the extracellular matrix in a biologically latent inactive form in which TGF-beta is caged in latency-associated peptides. The binding of alphaVbeta8 and alphaVbeta6 integrins to the RGD motif in latency-associated proteins leads to conformational changes in the cage structure. Subsequently, in cooperation with mechanical forces imposed on the extracellular matrices, a biologically active form of TGF-beta is released.

Concluding Remarks

Crosstalk between integrins and NO has been previously described. NO-mediated protein S-nitrosylation represents an important mode by which the post-translational modification of molecular function occurs [13,104]. In platelets, integrin alphaIIb/beta3 has been shown to be S-nitrosylated upon exposure to NO donors. This modification favors an inactive integrin state [105]. In addition to the integrin itself, NO induces protein S-nitrosylation of those cytoplasmic molecules important for integrin functions such as actin [106] and calpain [107]. S-nitrosylated short actin exhibits enhanced binding to the signaling scaffold protein VASP (vasodilator-stimulated phosphoprotein), thereby increasing actin polymerization, which modifies integrin-mediated cell adhesion [106]. S-nitrosylation of a protease calpain suppresses its ability to cleave talin [107], potentially inhibiting integrin activation. Conversely, integrin signaling modifies NO production. Activation of integrin alpha9/beta1 stimulates iNOS, thereby increasing NO production [108]. Signaling through integrin and integrin-linked kinase has been shown to regulate the expression of iNOS [109].

As NO is a short-lived molecule that can travel only a short distance, it effectively interacts only with neighboring cells within the same microenvironments [110]. In order for glia-produced NO to reach the appropriate target cells, glial cells must be positioned at the right location and at the right time. Integrins are thought to regulate glial migration to, and contact with, target cells, thereby playing a critical role in positioning glial cells. Cooperation and crosstalk between NO and integrins have been investigated in platelets [105], neutrophils [106], and endothelial cells [108,109], but not in glial cells. Therefore, it is imperative that future studies are carried out in order to address how the interplay between NO and integrins impacts on glial cell functions and on the progression of pathological pain.

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