

Possible Sites of Therapeutic Action in Restless Legs Syndrome: Focus on Dopamine and $\alpha_2\delta$ Ligands

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Key Words

Restless legs syndrome · Iron · Dopamine · Voltage-gated calcium channel $\alpha_2\delta$ ligands · Adenosine A_{2A} receptor · Opioids

Abstract

Restless legs syndrome (RLS) is a common sensorimotor disorder characterized by abnormal sensations that occur primarily at rest or during sleep, which are alleviated by movement of the affected limb. The pathophysiology of RLS remains unclear, although roles for dopamine dysfunction and brain iron deficiency have been proposed. The hypothalamic A11 dopaminergic circuit is used to explain the dopamine dysfunction in RLS and the potential therapeutic actions of dopamine D₂ agonists. Modulation of central and peripheral neuronal circuits may also explain the potential therapeutic sites of action of opioids, adenosine receptor ligands, and voltage-gated calcium channel $\alpha_2\delta$ ligands in RLS. The known and possible therapeutic benefits of these agents and their relationship to dopaminergic dysfunction in RLS are discussed in this review. Copyright © 2011 S. Karger AG, Basel

Introduction

Restless legs syndrome (RLS) is a common disorder of the nervous system, with a prevalence of 2.1–10.0% of the overall population [1, 2]. RLS is characterized by focal dysesthesias in the legs, otherwise referred to as abnormal limb sensations [3]. The characteristic hallmarks of RLS are sensations ranging from uncomfortable to irritating to painful. These sensations usually develop in the lower limbs together with an urge to move the affected limbs, and are felt mostly at rest and especially when attempting to sleep [3].

The clinical symptoms of RLS were first described in 1685 [4], with the term RLS introduced in 1945 by Karl Axel Ekbom [5, 6]. Two forms of RLS have been identified: primary and secondary. The primary, or idiopathic, form has a strong familial inheritable component [3] and typically appears around the mid-to-late twenties with a slow onset, although symptoms can begin at any age. The disease can fluctuate, but spontaneous long-term remissions are not usually observed in those patients with more advanced stages of the disorder. Symptoms typically worsen with increasing age, especially in patients with onset in young adulthood [3].

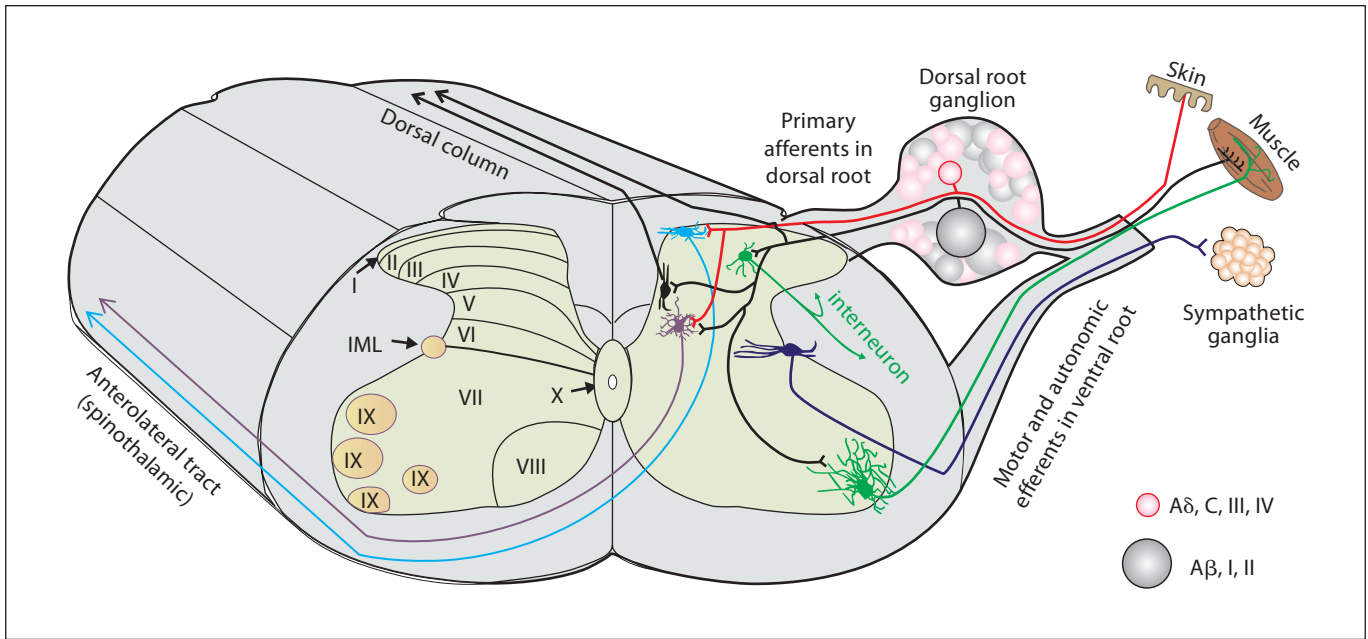


Fig. 1. The spinal cord is the gateway for information transfer between body and brain. As shown in the left half of the figure, the spinal cord is divided into anatomical layers or laminae that are divided into sensory dorsal horn (laminae I–VI), the intermediate gray (lamina VII), and ventral horn (laminae VII–IX). Laminae IX contains the various motoneuron pools (nuclei) whose axons, along with sympathetic (thoracic) or parasympathetic (sacral) preganglionic neurons located in the intermediolateral nucleus, exit via ventral roots. Sensory afferents with cell bodies located in

the dorsal root ganglia project onto spinal neurons largely within the dorsal horn. Sensory afferents transmit information from receptors in the periphery for pain, temperature, and crude touch ($A\delta$, C, III, and IV, axons in red in the online version) and non-pain encoding, stretch-sensitive, and force-sensitive mechanoreceptors ($A\beta$, I, and II, axons in black). Axon tracts of the dorsal column and anterolateral tract transmit sensory information to the brain. IML = Intermediolateral nucleus. Adapted from [51]. Copyright 2007, with permission from Elsevier.

Although first identified over 300 years ago as a disorder of the nervous system with spinal cord involvement [4], the cause of RLS is still not completely known. To date, 2 predominant mechanisms underlying RLS have been proposed in some detail, namely (i) dopaminergic dysfunction [7–9] and (ii) brain iron deficiency [10–12]. As such, pharmacological intervention to date has been focused on these 2 physiological processes. Furthermore, the complex nature of RLS as a somatosensory network disorder [9] suggests that several levels of interactions occur between central and peripheral circuits in parallel [8], many or all of which may be amenable to modulation by dopamine or iron levels.

Purpose and Perspective

The aim of this review is not to delve into the possible biological causes of RLS, but rather to summarize current therapeutic approaches for the treatment of RLS, be they

traditional pharmacological approaches or investigational agents. In addition, an appraisal of the mechanistic understanding of dopaminergic intervention forms the foundation of the proposed functioning of adenosine analogs, opioids, and $\alpha_2\delta$ ligands that are potential treatments for this disease (none are currently approved for the treatment of RLS). While numerous recent articles have highlighted the links between genetics and RLS and the role of brain iron deficiency in RLS [12, 13], these areas are beyond the focus of this review. Here, we examine the actions of other drug classes, including $\alpha_2\delta$ ligands, opioids, and adenosine-receptor ligands, and how these might be related to the effects of dopamine agonists in RLS. We posit that the therapeutic actions of D_2 -like agonists occur within the spinal cord and within associated peripheral afferent and efferent signaling systems (fig. 1). Using the hypothalamic A11 dopaminergic circuit as a platform, we explain the potential therapeutic actions of dopaminergics, opioids, adenosine analogs, and calcium channel $\alpha_2\delta$ ligands (fig. 2). It is important to note that the hypothalamic

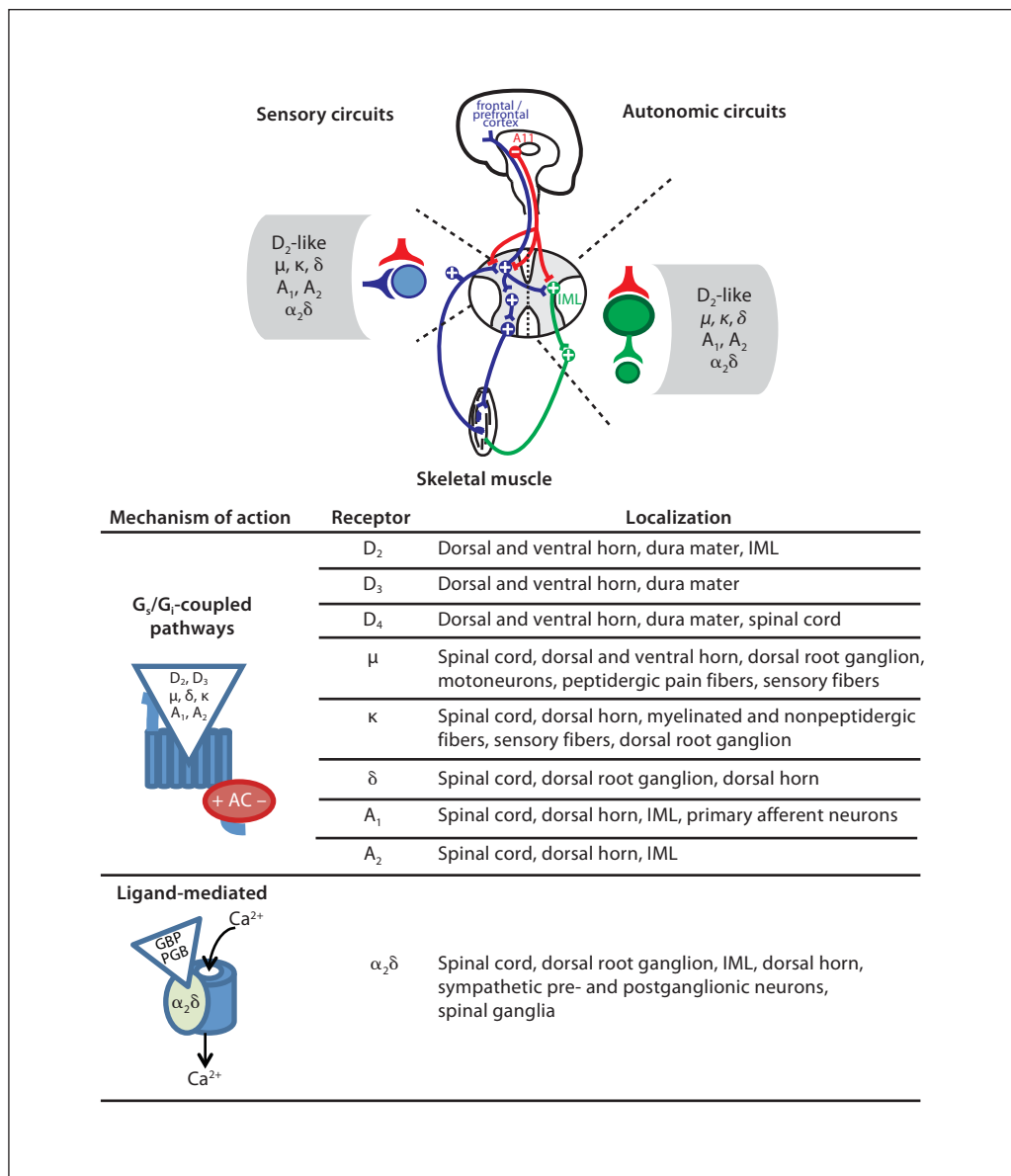


Fig. 2. Common targets of dopamine-, adenosine-, and opioid-receptor ligands in the proposed spinal/peripheral circuitry affected by dopamine. Based on the hypothesis detailed by Clemens et al. [8], dopaminergic projections descending from the A11 region of the hypothalamus project to spinal neuronal systems whose dysfunction leads to the RLS phenotype. Here, hypothalamic A11 dopaminergic neuron projections to sensory and autonomic regions of the spinal cord are shown (red). On the somatic side (left, in blue), sites for the effects of dopamine, adenosine A₁, adenosine A₂, and opioid receptors have been identified and are found in the dorsal horn. On the autonomic side (right, in green), actions of dopamine and adenosine receptors have been identified on sympathetic preganglionics, and there is evidence of opioid action. α₂δ ligands are proposed to exert actions in central circuits. Note that the effects of dopamine, adenosine, and opioid

receptor pathways alter levels of cAMP through G_i- or G_s-coupled control of adenylyl cyclase (AC), whereas α₂δ ligands are proposed to modify the excitability of the target tissues directly by binding to voltage-gated calcium channels. D₂-like, opioid, and A₁ receptors act through G_i to inhibit AC and A₂ receptors couple to G_s to stimulate AC. Receptors/binding sites that have been shown to be expressed or function within each circuit are listed in the grey boxes (*italics* indicate less evidence). Dopamine receptors, D₂: [54–56, 59]; D₃: [54, 55, 57]; D₄: [54, 55, 58]. Opioid receptors, μ: [89–91, 94, 95, 97–103]; κ: [89–91, 93–95, 102, 103]; δ: [89–92, 94, 96, 104]. Adenosine receptors, A₁: [112–115, 117–122, 124, 126, 128]; A₂: [112, 113, 115, 117, 123, 125]. α₂δ subunits: [116, 142, 144, 145, 147, 150–152]. GBP = Gabapentin; IML = intermediolateral nucleus; PGB = pregabalin. Adapted with permission from [8]. Colors refer to the online version only.

lamic A11 dopaminergic circuit, with its projections to the spinal cord, provides the only circuit proposed to date whose dysfunction would be consistent with both an RLS phenotype and an explanation of D₂-like therapeutic actions [8, 14]. While alternate models may conceivably exist that account for the dopamine hypothesis in RLS (and the beneficial actions of dopaminergics as therapeutics), to our knowledge, none have been proposed that clearly link circuit dysfunction to the RLS phenotype. Although changes in central nervous system (CNS) dopaminergic function have been identified in other regions in association with RLS, including iron metabolism [10, 12, 15, 16], their correlation may be a secondary consequence of RLS rather than a primary causal factor.

Current Treatment Approaches to RLS

Dopamine Receptor Agonists

Dopaminergic drugs have shown efficacy as an RLS treatment in numerous large-scale clinical studies [17], and dopamine agonists improve RLS symptoms from the first day of application [18]. Currently, dopamine agonists targeting D₂-like receptors (most of the current agonists have the highest affinity for D₃ receptors) represent the first line of pharmacological intervention [19]. Clinical studies have shown that levodopa, pramipexole, ropinirole, lisuride, pergolide, cabergoline, and rotigotine reduce the symptoms of RLS [17]. Among these, pramipexole, ropinirole, and rotigotine are approved to treat RLS in various countries. All 3 have the highest affinity for D₃ receptors [20–22]. However, long-term use of dopamine therapy in patients with RLS has potentially serious consequences, including symptom rebound, development of compulsive disorders, and augmentation. Augmentation is the paradoxical emergence of more severe RLS symptoms, including worsening of symptoms, symptoms appearing earlier in the day, and spread of symptoms to previously unaffected body parts [9, 23, 24]. Furthermore, the ergot-derived dopamine agonists (e.g. pergolide and cabergoline) are generally not recommended for the treatment of RLS because of the rare, but serious, potential for pulmonary and cardiac fibrotic adverse events [17]. Because RLS is typically a life-long condition, alternative therapeutic targets for modulating sensory perception in RLS have been sought.

Iron

The association between iron deficiency and RLS points toward iron supplementation as a therapeutic op-

tion in patients with low iron levels. In one study, oral iron showed no benefits on RLS symptoms [25]; however, another small study of oral iron therapy that used the validated International Restless Legs Study Group Severity Scale (IRLS) showed an improvement in RLS symptoms in patients with RLS and low-normal serum ferritin levels [26]. High-dose iron dextran infusion has been shown to transiently reduce RLS symptoms in patients with end-stage renal disease or with low ferritin levels [27, 28]. Intravenous iron dextran also reduced the symptoms of RLS in patients refractory to other treatments; however, the duration of effect was variable and the results of treatment were not predictable from patients' baseline characteristics [29]. Taken together, the clinical evidence suggests that restoration of serum ferritin levels with oral iron therapy may benefit some patients with RLS [30]. Adverse events of iron therapy include constipation, nausea, a potential for iron overload, and anaphylactic reactions to iron dextran [25, 29].

Opioids

Low- and high-potency opioids, including oxycodone, tramadol, and methadone, have shown efficacy in reducing the symptoms of RLS in short-term studies [31–33]. Opioids are frequently used off-label in patients with RLS, particularly in those refractory to dopamine agonists; however, large controlled clinical studies establishing their efficacy in RLS are lacking. Thus, opioids are typically considered only second-line treatment to dopamine agonist therapy [17, 34, 35]. Augmentation has been reported following long-term treatment with tramadol [36], along with other safety concerns with opioids, including constipation, respiratory depression, sleep apnea, and the potential for addiction [17, 37, 38]. The addiction potential of opioids coupled with their controlled regulatory status have limited their commercial development for RLS [17].

Possible Alternate Approaches

Adenosine Receptor Antagonists

Adenosine receptors have recently been proposed as potential therapeutic targets for RLS [39, 40]. However, despite the putative links between adenosine and dopamine in motor and sensory control and their common second messenger targets, no clinical trials of adenosine receptor ligands for the treatment of RLS have been reported. Therefore, the efficacy, safety, and validity of adenosine receptor-blocking drugs as viable, novel therapies for RLS have not been established.

Clinical Efficacy of $\alpha_2\delta$ Ligands in Controlling the Symptoms of RLS

Recent clinical studies have shown efficacy for gabapentin, gabapentin enacarbil, and pregabalin in reducing the sensorimotor symptoms of RLS, although none of these agents are approved for the treatment of RLS. In a small placebo-controlled study, gabapentin improved RLS symptoms, periodic leg movements during sleep, and sleep architecture in patients with RLS, most of whom had the idiopathic form [41]. Gabapentin enacarbil, a prodrug of gabapentin with improved pharmacokinetics [42], significantly improved IRLS scores, clinician-rated response to therapy, and some sleep parameters in patients with idiopathic RLS [43–46]. In a small uncontrolled study of pregabalin in clinical practice, most patients with secondary RLS and neuropathic pain reported improvements in their RLS symptoms [47]. More recent randomized, double-blind, placebo-controlled studies of pregabalin have demonstrated significant improvements in IRLS scores [48, 49], periodic leg movements during sleep [48], and sleep architecture [48] in patients with idiopathic RLS. Common side effects of $\alpha_2\delta$ ligands in patients with RLS include malaise, somnolence, dizziness, headache, dry mouth, and fatigue [41, 43, 44, 46, 48, 49]. Unlike dopamine agonists, symptom rebound has not been observed following withdrawal of pregabalin or gabapentin enacarbil [49, 50].

Potential Therapeutic Sites of Action

RLS is a disorder characterized by abnormal sensations. Therapeutically, there are multiple sites for the control of aberrant somatosensory processing prior to perception in the cortex. Those associated with spinal cord circuits are described below.

A11 Dopamine, Dopamine Receptors, and Spinal Circuits in Relation to RLS

A concise overview of spinal cord function and its functional systems can be found in Hochman [51]. Briefly, the spinal cord is the part of the CNS that provides the gateway for information transfer between body and brain (fig. 1). It is also an integrative center for neuronal circuits that coordinate complex sensory, motor, and autonomic functions. The entire sympathetic neural output departs the CNS via convergence onto spinal sympathetic preganglionic neurons, and the entire somatosensory input from the body is first processed through the spinal cord. Moreover, the entire neural circuitry required to generate

locomotion and other complex movements resides entirely within the cord.

Spinal cord function is strongly modulated by serotonin, norepinephrine, and dopamine. Dopamine is a neurotransmitter that binds to D₁-like (D₁ and D₅) receptors coupled to the G protein G_s to stimulate adenylyl cyclase and increase formation of cyclic adenosine monophosphate (cAMP), leading to a facilitation of network excitability. It also binds to D₂-like (D₂, D₃, and D₄) receptors coupled to G_i to inhibit adenylyl cyclase and cAMP production, generally depressing network function [52]. Dopamine and all 5 dopamine-receptor subtypes can be found in the rodent spinal cord, including the dorsal and ventral horn and dura mater (fig. 2) [53–59]. Furthermore, D₂ receptors have been found in the rodent intermediolateral cell column, the location of the sympathetic preganglionic neurons [56]. In comparison, in the non-human primate lumbar spinal cord, mRNA for D₂, D₃, and D₅ receptors was detected with *in situ* hybridization without evidence of D₁ mRNA expression [60]. While this does not exclude the presence of spinal cord D₁ receptor protein in primary afferent terminals, this study highlights the presence of interspecies differences in expression. Overall, dopamine appears to have lower affinity for the D₁-like receptors compared with the D₂-like receptors, and the highest affinity for D₃ receptors [8, 61].

A small cluster of dopaminergic neurons (A11 population) found in the dorsal-posterior hypothalamus [8, 60, 62] is thought to represent the sole source of spinal dopamine in the rat [63] and the predominant source in the mouse [64]. A11 projections issue collaterals throughout the rostrocaudal extent of the spinal cord [63]. As collaterals project to sensory, motor, and autonomic regions [53, 60], it is likely that there could be coordinate regulation of these spinal functional systems, and consequently, a coordinate dysregulation if A11 drive was altered.

Dysfunction of the A11 region has been implicated in the pathophysiology of RLS [8, 14, 60, 62]. In RLS, it is possible that the observed circadian cycling of dopamine that reaches its lowest levels in very early morning [65] is either excessively lowered (hypofunction) or has a blunted reduction (hyperfunction). In such a push-pull mechanism [9], the hypothesis of A11 hypofunctioning at nighttime in RLS assumes low dopamine release has inhibitory actions that would normally preferentially activate high-affinity D₂-like receptors to reduce sensory responsiveness. The consequence of a hyporelease of dopamine then has been hypothesized to lead to a series of parallel events that disinhibit spinal cord sensory, motor, and autonomic systems [8, 60]. Nighttime hypofunction could arise from in-

creased circadian amplitude fluctuations with even lower dopamine levels at night [65]. Another possibility is that neurodegenerative loss of spinal dopaminergic terminals from the A11 region results in a decrease of dopamine in the spinal cord [66]. While loss is possible at the level of terminals in the spinal cord, as in the nigrostriatal dopamine system, there does not appear to be any loss of tyrosine hydroxylase-staining cell bodies in the A11 region from postmortem tissue of patients with RLS [67].

An alternate hypothesis is that the principal pathophysiology in RLS resides in a hyperfunctioning of the A11 dopaminergic pathways at nighttime. Dopamine levels fluctuate in the CNS in a circadian manner, and there is an age-dependent blunting of circadian amplitudes with age [68, 69]. The weakened reduction and hence higher levels of dopamine at night could result in an aberrant activation of lower affinity, but 'excitatory' drive mediated by D₁ or D₅ receptors, even when low levels of the receptor are present [60]. Indeed, there is evidence of an altered circadian rhythmicity in dopamine metabolism in RLS [70], with an intriguing blunting of and inversion in circadian tyrosine hydroxylase expression in the sympathetic preganglionic neurons of D₃-knock-out (D₃KO) mice [71]. As microdialysis studies have demonstrated that spinal dopamine levels significantly decreased during rapid eye movement sleep when compared with wakefulness [72], an increased nighttime dopamine drive could aberrantly facilitate sensorimotor systems in RLS – this is consistent with the emergence of periodic leg movements and increased reflex excitability in sleep [73, 74].

All dopamine receptor subtypes are also present on afferent neurons within cell bodies in the dorsal root ganglia [75], with the highest dopamine affinity and greatest inhibitory actions in the small diameter neurons [76], and pain transmission is selectively inhibited by D₂-like agonists [77]. Assuming that RLS is primarily a sensory disorder, *this action alone may explain the therapeutic actions of dopaminergics completely independent of any need for central dopamine dysfunction.* This would be consistent with the apparent absence of D₂-like autoreceptor function on descending dopaminergic terminals [78]. The possibility of a spinal site of action is further provided by studies with the D₃KO mouse. The D₃KO mouse displays hyperactivity, increased locomotor activity, and hypertension [79, 80]. This phenotype resembles features of patients with RLS [8, 81]. Moreover, a recent study showed that both iron deficiency and D₃ receptor dysfunction can mimic evoked sensory and motor symptoms in mice resembling those observed in RLS patients [82]. In the isolated spinal cord of the D₃KO mouse, the

effect of dopamine on spinal reflexes is converted from inhibitory to mildly facilitatory, showing that D₃ receptors are involved in limiting spinal cord sensory processing [83]. Further studies are warranted to confirm these hypotheses in humans, especially in light of the apparent species-specific expression of dopamine receptor subtypes in the spinal cord [60]. Independent of the A11 being dopaminergic, as discussed above, or possibly only L-dopa-ergic [60, 78, 84], there is little doubt that A11 stimulation produces spinal antinociceptive actions via D₂-like receptor selective mechanisms [77, 84, 85].

Mechanistic Appraisal of Potential Pharmacological Intervention beyond Dopamine D₂ Agonists

Opioid Receptors in RLS

As is the case for D₂-like receptors, activation of opioid receptors, which include the μ , δ , and κ subtypes, results in G_i-coupled inhibition of adenylyl cyclase and cAMP formation in the postsynaptic neuron [86]. This signaling pathway mediates suppression of voltage-gated calcium currents and activation of outward potassium currents, resulting in hyperpolarization, reduced neurotransmitter release, inhibition of dorsal horn neurons, and depression of excitatory postsynaptic potentials [87, 88]. μ , δ , and κ opioid receptors are found in the spinal cord [89–93], in the dorsal and ventral horn [94–101], including on intrinsic neurons of the dorsal horn [87]. Opioid receptors are also expressed presynaptically on C-fiber primary afferents and in dorsal root ganglia (fig. 2) [90, 91, 102–104]. Expression of opioid receptors on sympathetic preganglionic neurons has been hypothesized [105], and there is evidence for it in the human spinal cord [106]. A rationale for opioid dysfunction in RLS has been proposed based on positron emission studies, which show reduced opioid-receptor availability in the medial affective pain system in the brain of patients with RLS and an inverse relationship between opioid receptor availability and severity of RLS [107]. Reduced opioid receptor availability may be a result of higher receptor occupancy by endogenous opioids, presumably via increased endogenous release of opioids, or opioid receptor downregulation and/or sequestration [107].

There are some data to support the concept that opioids improve the symptoms of RLS via indirect effects on the dopamine system [108]. Postsynaptic opioid receptor blockade with the opioid receptor antagonist naloxone reversed the effects of opioids but not dopamine agonists on RLS symptoms, while postsynaptic dopamine receptor blockade with dopamine receptor antagonist pimozone re-

versed the effects of both dopamine agonists and opioids [108]. Taken together, available data suggest that the beneficial effects of opioids in RLS may be mediated by dopaminergic neurotransmission and thus not related to specific deficiencies of the endogenous opioid system [109].

Adenosine Receptors in RLS

Adenosine has been shown to play a role in motor activity and nociception [110, 111], and several adenosine receptor subtypes (A_1 , A_{2A} , A_{2B} , and A_3) are known to have opposing actions on cAMP formation; A_1 receptors couple with G_i to inhibit and A_2 receptors to G_s to stimulate formation of cAMP [112]. A_1 and A_2 receptors are expressed in the spinal cord [112–116], in dorsal horn neurons [114, 117–124], and the intermediolateral cell column (fig. 2) [125, 126]. A_1 receptors are found in both excitatory and inhibitory cell bodies and processes in the inner part of lamina II of the dorsal horn, with lesser expression in primary afferent neurons [120].

Activation of the A_{2A} receptor increases inhibitory neurotransmission onto sympathetic preganglionic neurons and interneurons of the intermediolateral nucleus, while activation of the A_1 receptor decreases excitatory neurotransmission onto the same neurons [125]. Thus, activation of both A_{2A} and A_1 receptors would presumably result in inhibition of the postsynaptic neuron [125]. Similarly, activation of A_1 and A_2 receptors mediates spinal antinociception in an animal model of pain [127], and an A_1 receptor agonist reduced hyperalgesia following spinal cord injury [128]. Additionally, A_{2A} receptors have been implicated in the development of neuropathic pain [129].

A functional link between adenosine and dopamine receptors has been reported in the brain [111]. In the striatum, A_{2A} receptors form heterodimers with D_2 receptors and may modulate dopaminergic activity by reducing the affinity of D_2 receptors for dopamine, inhibiting D_2 -mediated signal transduction and presumably leading to modulation of striatal glutamatergic neurotransmission [39]. A_{2A} receptor antagonists could, therefore, potentiate the effects of D_2 receptor agonists [39]. Similar interactions may occur elsewhere in the CNS including the spinal cord. More recently, data suggest that iron deficiency induces a functional upregulation of both striatal pre- and postsynaptic A_{2A} receptors [40].

The $\alpha_2\delta$ Calcium Channel Subunit and the Effects of Its Ligands on Sensory and Motor Neuromodulation

Voltage-gated calcium channels are multisubunit complexes composed of pore-forming α_1 subunits and auxiliary $\alpha_2\delta$, β , and γ subunits that regulate calcium

flow into cells [130]. The α_1 pore regulates passage of calcium into the cell, while the auxiliary subunits serve to modulate calcium channel expression and function [130]. Gabapentin and pregabalin are structurally analogous to the neurotransmitter gamma aminobutyric acid (GABA). Although several studies have shown agonist activity for gabapentin at $GABA_B$ receptors in a mouse pituitary intermediate melanotrope cell line [131], cortical slices [132], hippocampal neurons [133], and at recombinant $GABA_B$ receptors expressed in *Xenopus laevis* oocytes [133], others have failed to reproduce these findings and gabapentin and pregabalin do not appear to bind with high-affinity binding to these receptors [134–137]. Accumulating preclinical evidence indicates that gabapentin and pregabalin are selective, high-affinity ligands for the calcium channel $\alpha_2\delta$ subtype-1 protein and their effects are likely independent of $GABA_B$ receptors [130, 134]. The functional consequences of binding to the $\alpha_2\delta$ protein have been evaluated in multiple systems and in vivo systems [134, 138–140]. Such studies have pointed to a modulation in calcium flux at the nerve terminal in response to membrane depolarization that would result in a decrease in the release of excitatory neurotransmitters, such as glutamate and substance P [134, 141]. Consistent with this hypothesis, a number of groups have demonstrated such effects of $\alpha_2\delta$ ligands on neurotransmitter release [130, 134, 141]. Gabapentin and pregabalin were also shown to reduce trafficking of $\alpha_2\delta$ subunits to the plasma membrane of presynaptic nerve terminals that results in fewer functional channels at the nerve terminal, which may be another mechanism by which $\alpha_2\delta$ ligands affect neurotransmitter release [130, 142, 143]. While these results followed chronic application of these drugs, it is conceivable that some of the immediate effects of $\alpha_2\delta$ ligands may be mediated by altered protein trafficking.

Unlike dopamine-, opioid-, and adenosine-receptor ligands, $\alpha_2\delta$ ligands do not require G-protein-coupled, cAMP-mediated second messenger pathways to exert their effects on voltage-gated calcium channels. $\alpha_2\delta$ -containing calcium channels are found at various locations within the proposed RLS circuit (fig. 2), including the dorsal horn [144], dorsal root ganglia neurons [139, 142, 144–149], intermediolateral neurons [150], and sympathetic pre- and postganglionic neurons [151, 152]. Thus, they are in a prime position to control the excitability of the circuitry at the various points that may be involved in the symptomatology of RLS. Sympathetic spinal ganglia also express $\alpha_2\delta$ subunits [152]. Furthermore, expression of presynaptic $\alpha_2\delta$ subunits is induced by injury [146] and $\alpha_2\delta$ ligands inhibit voltage-gated channel activity in dor-

sal root ganglia neurons [138] and reduce sympathetically maintained pain at the spinal level [151].

It is hypothesized from the results of preclinical studies that the binding of pregabalin to the $\alpha_2\delta$ subtype-1 protein causes a decrease in the release of synaptic vesicles at a variety of different synapses, including those that utilize substance P, calcitonin gene-related peptide, and glutamate, which reduces subsequent postsynaptic neuronal firing. In certain disease states, such as neuropathic pain, abnormally intense neuronal firing is evident and this may be the case in RLS at the level of specific regions of the dorsal horn. Afferent overactivity could drive synaptogenesis, resulting in amplification of excitatory neurotransmission and overwhelming descending inhibition. More recent findings suggest that gabapentin and other $\alpha_2\delta$ ligands may act on neuronal processes that do not directly involve voltage-gated calcium channels such as the reduction of the expression of genes in the NF- κ B pathway [153]. In addition, gabapentin was shown to block thrombospondin-mediated excitatory synapse formation in vitro and in vivo, which suggests that $\alpha_2\delta$ subtype-1 may function in synaptogenesis [154].

A recent series of articles has also proposed modulation of descending noradrenergic inhibitory pathways by gabapentin and pregabalin. It has been proposed from these preclinical studies that descending noradrenergic inhibitory pathways are facilitated by these 2 $\alpha_2\delta$ ligands. These data suggest an additional direction in the mechanism of action of gabapentin and pregabalin in pain, and that their proposed spinal site of action includes amplification of descending inhibitory controls [155–158]. Pregabalin is proposed to reduce hyperexcitability in neuronal networks by binding to the $\alpha_2\delta$ protein and subsequently reducing neurotransmitter release, inhibiting aberrant excitatory synapse formation, and/or modulating descending inhibition. These may be the mechanisms by which pregabalin serves to modify the sensory-related symptoms of RLS and thus relieves the focal dysesthesias and the subsequent motor movements and sleep deprivation.

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Discussion

RLS is a neurological disorder that has been studied for over 3 centuries. The known modulatory actions and sites of action of dopamine on spinal cord function can be used to physiologically explain the mechanisms of action of several potential therapies for RLS. We provide evidence that the potential therapeutic actions of D₂-like agonists, opioids, and adenosine A_{2A} antagonists converge on the common second messenger pathway of regulating G-protein-coupled cAMP formation, which results in neuronal hyperpolarization and reduced excitability. While localization of $\alpha_2\delta$ -binding sites overlaps with those of D₂-like receptor agonists, $\alpha_2\delta$ ligands directly target voltage-gated calcium channels and thus dampen sensory neurotransmission through reductions in excitatory neurotransmission. It is conceivable then that these new classes of pharmaceutical drugs may herald additional approaches that can relieve the symptoms of RLS.

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Note Added in Proof

In April 2011 after acceptance of the manuscript, the U.S. Food and Drug Administration approved Horizant Extended Release Tablets (gabapentin enacarbil) for the treatment of moderate-to-severe primary Restless Legs Syndrome (RLS) in adults.

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