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Adenosine monophosphate-activated protein kinase (AMPK) activators for the prevention, treatment and potential reversal of pathological pain

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Abstract

Pathological pain is an enormous medical problem that places a significant burden on patients and can result from an injury that has long since healed or be due to an unidentifiable cause. Although treatments exist, they often either lack efficacy or have intolerable side effects. More importantly, they do not reverse the changes in the nervous system mediating pathological pain, and thus symptoms often return when therapies are discontinued. Consequently, novel therapies are urgently needed that have both improved efficacy and disease-modifying properties. Here we highlight an emerging target for novel pain therapies, adenosine monophosphate-activated protein kinase (AMPK). AMPK is capable of regulating a variety of cellular processes including protein translation, activity of other kinases, and mitochondrial metabolism, many of which are thought to contribute to pathological pain. Consistent with these properties, preclinical studies show positive, and in some cases disease-modifying effects of either pharmacological activation or genetic regulation of AMPK in models of nerve injury, chemotherapy-induced peripheral neuropathy (CIPN), postsurgical pain, inflammatory pain, and diabetic neuropathy. Given the AMPK-activating ability of metformin, a widely prescribed and well-tolerated drug, these preclinical studies provide a strong rationale for both retrospective and prospective human pain trials with this drug. They also argue for the development of novel AMPK activators, whether orthosteric, allosteric, or modulators of events upstream of the kinase. Together, this review will present the case for AMPK as a novel therapeutic target for pain and will discuss future challenges in the path toward development of AMPK-based pain therapeutics.

INTRODUCTION

Pain is the most common reason that people seek medical attention. While acute pain serves an important survival function, pain can sometimes outlive its protective attributes and become pathological (1, 2). Up to a third of the population of most developed countries suffer from what can be categorized as pathological pain (3). While effective therapeutics are available for many forms of acute pain, once pain enters a pathological state, a state which can last for months to years, very few efficacious therapeutics are currently available (3). Moreover, those treatments that do show limited efficacy primarily target mechanisms

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that palliatively reduce pathological excitability in the peripheral or central nervous systems (e.g. antiepileptics) and do not modulate the underlying processes that cause these systems to become hyperexcitable. Disease modifying approaches to treat pathological pain are needed to meet the challenge posed by this medical problem.

There are several possible ways to approach the problem of developing disease modifying treatments for pathological pain. An important first step in this process is to understand the underlying molecular causes for the development and maintenance of pathological pain. While these molecular events are still not completely elucidated, major advances have been made in this area using preclinical pain models (4). An important principle that has emerged from this work is that pathological pain is caused by plasticity in both the peripheral and central nervous systems (1, 2, 5–12). Here we will focus mainly on plasticity in the peripheral nervous system and how this can be targeted with adenosine monophosphate-activated protein kinase (AMPK) based therapeutics (13). We will make the case that AMPK activators may represent the first disease-modifying agents for pathological pain highlighting both the rationale behind this premise and what therapeutic strategies might best be employed to engage AMPK in this therapeutic setting.

MECHANISMS OF PAIN PLASTICITY IN THE PERIPHERAL NERVOUS SYSTEM: A KEY ROLE FOR TRANSLATION CONTROL

Injury to peripheral tissues and/or peripheral nerves changes the sensitivity of nociceptive afferent neurons such that they become hyperexcitable. This change in the sensitivity of nociceptors occurs rapidly after injury and can be mediated by a broad variety of endogenous factors that act on receptors expressed by nociceptors (1, 5, 6, 10, 11, 14, 15). Immediate changes in excitability are generally attributed to short term signaling mediated by α subunits of G-protein coupled receptors (GPCRs) or via activation of kinases downstream of tyrosine kinase receptors (Trks). In many cases these short-term changes in the sensitivity of nociceptors resolve once the stimulus causing the signaling events to occur is removed. However, in cases where pain becomes pathological this may not be the case. One possible explanation for how this occurs is that certain signaling events are capable of causing long-term changes in the function or phenotype of the nociceptor causing some semi-permanent alteration in pain sensitivity (15, 16). It is likely that this transition to a pathological pain state is controlled by changes in gene expression, either at the level of transcriptional (7, 17–20) or translational changes (10, 13, 21). In fact there is abundant evidence that both of these forms of gene expression control are crucial for pathological pain but this review will focus on translation control due to its direct link to AMPK.

Neurons are the largest cells in the body with axons that can cover distances of up to a meter in humans. Among all neurons in the body, peripheral neurons, including nociceptors, are among the largest with their cell bodies found either in the dorsal root ganglion (DRG) or trigeminal ganglion (TG) and their axons extending out to target tissues including the skin, viscera, bones and ligaments. Plasticity is a key feature of the nervous system and multiple lines of evidence indicate that a critical molecular event for the induction and maintenance of plasticity in neurons is the local translation of nascently synthesized proteins in distal

dendrites or axons (22–27). This process has been linked to learning and memory in the central nervous system (CNS) and we will review evidence that it is linked to pathological pain in the peripheral nervous system (PNS). Local translation at distal sites (dendrites or axons) in neurons requires three major features: 1) the presence of ribosomes at distal sites in these cells, 2) the transport of mRNAs to these sites and 3) the presence of signaling machinery linking extracellular events to intracellular translation. In the CNS these criteria are satisfied at the level of dendritic spines where molecular changes involved in learning and memory are thought to occur (23–25, 27). In the PNS, including in nociceptors, there is now strong evidence that these criteria are also met (22) and that they are altered in situations where pathological pain emerges (10, 13, 21).

The original view of translation of new proteins in the PNS was that this solely occurred in the soma of DRG or TG neurons (28). This idea was based on the limited number of ribosomes found in adult DRG axons. It is now well accepted that local translation in DRG neuron axons is an important component of development, especially at the growth cone of axons during pathfinding to target tissues. Following development, there are indications that local translation in axons is decreased, however, these axons, and potentially even their peripheral terminals, still contain ribosomes, mRNAs, mRNA trafficking proteins and signaling molecules involved in translation control (22, 28–33). Work in the area of pathological pain plasticity has mostly focused on these signaling molecules with the major focus falling on a kinase called mechanistic target of rapamycin complex 1 (mTORC1). mTORC1 is regulated by upstream activation of Trks and engagement of PI3K/AKT signaling. When mTORC1 is activated, it stimulates phosphorylation of a family of proteins that bind to the 5′m7GTP Cap structure (5′Cap) of mRNAs (34). A key event in this process appears to be phosphorylation of the eukaryotic initiation factor (eIF) binding protein (BP) 4EBP. When 4EBP is phosphorylated by mTORC1, 4EBP dissociates from eIF4E, the primary 5′Cap binding protein in all cells, allowing for more efficient association of eIF4E with eIF4A (a deadbox family helicase) and eIF4G (a scaffolding protein) (34). Collectively these three protein, eIF4E, eIF4A and eIF4G form what is called the eIF4F complex. This eIF4F complex is associated with efficient translation of target mRNAs and is an important regulatory complex for changes in mTORC1 activity (35, 36).

Multiple lines of evidence indicate that mTORC1 is linked to plasticity in nociceptors. First, rapamycin, a highly selective inhibitor of mTORC1, blocks plasticity induced by a variety of factors that act specifically on nociceptive neurons (37, 38). Moreover, rapamycin decreases the sensitivity of a subset of nociceptors thought to play an important role in mechanical hypersensitivity following injury (39, 40) and reduces neuropathic mechanical hypersensitivity in rats (41) and mice (42). Finally, factors that are well known to sensitize nociceptors, such as nerve growth factor (NGF), and peripheral nerve injury, which causes neuropathic pain, increase mTORC1 activity in nociceptive PNS neurons (37, 43) (Fig 1). In addition to its role in the PNS, a broad variety of studies have linked increased mTORC1 activity to plasticity in the spinal dorsal horn after peripheral injury (39, 44–49) or even exposure to drugs of abuse (50). Here mTORC1 appears to control changes in synaptic strength between nociceptors and CNS neurons that relay nociceptive information to the brain via changes in postsynaptic expression of plasticity-related genes. However, an alternative interpretation is that presynaptic effects in the dorsal roots predominate, as there

is evidence that mTORC1 activity at this site contributes to neuropathic pain (39). While more work is needed to understand these events in finer detail, it is clear that mTORC1 is a crucial molecular signaling hub for plasticity that underlies pathological pain.

MAPK represents another signaling pathway linking extracellular signals to translation in neurons. The MAPK isoform ERK, which plays a crucial role in nociceptor excitability and synaptic plasticity in the CNS, phosphorylates eIF4E at Ser 209 via its downstream kinase MAP kinase interacting kinase (MNK, (51) Fig 1). Two isoforms of MNK have been described and both are expressed in the nervous system (BioGPS.org). It remains to be seen if one of these isoforms plays a predominant role in control of translation in neurons but what is clear is that eIF4E phosphorylation regulates the translation of a subset of mRNAs. These mRNAs, referred to as “eIF4E strong” mRNAs, have been elucidated using mice where the Ser 209 site of eIF4E has been mutated to Ala rendering the protein unphosphorylatable (52). eIF4E strong mRNAs largely encode cytokines, chemokines and other plasticity related proteins (53). Recently MMP9 was identified as an eIF4E strong mRNA (54). Importantly multiple lines of evidence suggest that MMP9 expression in the DRG is regulated by injury and/or by opioids and is involved in pathological pain (55–58). ERK signaling to eIF4E is likely involved in the promotion of pain plasticity by endogenous factors that signal via ERK to induce pain hypersensitivity. One such example is interleukin 6 (IL6). IL6 exposure to DRG neurons increases ERK and eIF4E phosphorylation and promotes mechanical hypersensitivity in a translation-dependent fashion (37, 59–61) (Fig 1). Moreover, IL6 increases phosphorylation of the voltage gated sodium channel $Na_v1.7$ via an ERK-dependent process (62). It is not clear if ERK-mediated control of translation also regulates $Na_v1.7$ or $Na_v1.8$ expression, localization or phosphorylation (Fig 2A and B). However, several studies have shown that ERK and p38 MAPKs directly phosphorylate $Na_v1.7$ and $Na_v1.8$ leading to an increase in activity of this crucial voltage gated sodium channel causing enhanced nociceptive sensitivity (63–65).

The data discussed above point to mTORC1 and MAPK, especially ERK, signaling as prime targets for control of pathological pain with the potential for disease modifying effects. While a number of studies have indicated positive effects for the mTORC1 inhibitor rapamycin using an acute drug administration paradigm (37–40, 42, 49, 50, 61), longer term treatment with rapamycin causes an engagement of feedback signaling through p70S6 Kinase (p70S6K) in DRG neurons that leads to stimulation of ERK and nociceptor hyperexcitability (41). Several clinical studies suggest that rapamycin treatment in humans can promote pain hypersensitivity and rapamycin treatment has even been linked to devastating human pain disorders such as complex regional pain syndrome, albeit in limited numbers of patients (66–71). The agreement of these basic and clinical studies likely rule out the use of rapamycin as a pain therapeutic. MAPK inhibitors have been widely investigated as pain therapeutics (17) including some clinical trials for p38 inhibitors that have had mixed success (72). A larger number of clinical trials have been conducted in the cancer area using MAPK inhibitors and these trials have sometimes demonstrated that single kinase inhibitors, as is the case with mTORC1 (73, 74), can also lead to engagement of feedback signaling that limits their usefulness in certain patients (75–77). A possible alternative approach to this problem is the use of AMPK activators. This makes sense from a molecular signaling perspective because AMPK activation short circuits signaling in the

mTORC1 and MAPK pathways at points where feedback signaling is frequently engaged (e.g. at IRS-1, (78), Fig 1). In fact, in DRG neurons, AMPK activation mitigates the negative effects of rapamycin on nociceptor hyperexcitability via negative regulation of p70S6K feedback signaling to IRS-1 (41). Below we will discuss evidence that AMPK activators may represent some of the first pain therapeutics with the potential for disease modifying properties.

AMPK ACTIVATORS FOR NEUROPATHIC PAIN

Injury to the PNS, either through trauma, metabolic disease or via exposure to drugs, such as chemotherapeutics, can cause neuropathic pain. Neuropathic pain is generally characterized by hypersensitivity to mechanical stimulation, hypersensitivity to cold and ongoing burning pain (79). Traumatic injury to peripheral nerves in rats and mice causes activation of mTORC1 (43, 80) and MAPK signaling (43, 81–84) in DRG neurons and their axons. It is also alters the expression of upstream and downstream signaling components of these pathways (43), increases the expression of RNA binding and transport proteins (43) and stimulates the translocation of mRNAs to the axonal compartment (85–89). Coupled to these changes is an overall increase in the rate of translation in peripheral nerves suggesting a reorganization of the translational machinery into the axonal compartment of DRG neurons (43), a process that may be linked to regeneration after injury (22, 31), but these same molecular events may also cause changes in excitability in the injured DRG (43, 87).

AMPK activators (either metformin or A769662) administered to rats or mice following peripheral nerve injury reduce mechanical hypersensitivity starting 2 – 3 days after treatment (41, 43). Interestingly, these compounds induce a complete reversal of mechanical hypersensitivity within 7 days and after cessation of treatment mechanical hypersensitivity does not return, suggesting disease modification by the course of treatment. Seven-day treatment with metformin effectively reverses mechanical hypersensitivity after nerve injury when started at any time between 14 and 60 days after injury (41, 43). Moreover, mechanical hypersensitivity does not return after metformin treatment even months after treatment was stopped (41). Metformin treatment also normalizes increased translation rates in injured sciatic nerve (43). This finding lends further support to the notion of disease modifying properties of AMPK activators for neuropathic pain caused by traumatic injury to the PNS. Electrophysiogical studies on DRG neurons show that AMPK activators such as metformin and A769662 reduce the excitability of DRG neurons over a one-hour treatment time *in vitro* (43). While still untested *in vivo*, these findings may indicate that AMPK activators are capable of reducing ectopic activity in DRG neurons following injury. Since this event is thought to underlie spontaneous burning pain after nerve injury this suggests that AMPK activators can modify two key symptoms of neuropathic pain: mechanical hypersensitivity and spontaneous pain (79).

Exposure to chemotherapeutic agents for the treatment of cancer frequently causes a peripheral neuropathy that can be painful in many patients. This side effect is the most common dose-limiting factor for chemotherapeutic treatment and can have devastating consequences for patients (90). The mechanisms underlying chemotherapy-induced neuropathic pain (CIPN) are still not completely understood but they likely share some

properties with trauma-induced neuropathic pain due to overlapping symptomology and physiological findings such as epidermal nerve fiber dieback and ectopic activity in DRG neurons (91). Treatment of mice with chemotherapeutics causes a loss of tactile sensation and an accompanying mechanical hypersensitivity. Concomitant administration of metformin prevents the loss of tactile sensitivity and the development of mechanical hypersensitivity suggesting that activation of AMPK at the time of chemotherapeutic treatment may prevent CIPN (92). Importantly, treatment with metformin after the development of CIPN had no effect (92). This is in stark contrast to trauma induced neuropathic pain where metformin treatment resolved established mechanical hypersensitivity. Nevertheless, because metformin is actively being investigated as an adjuvant treatment for chemotherapeutics in cancer trials (93), this emerging treatment approach may have the added value of preventing the development of CIPN in this patient population. It cannot be overstated that this effect would eliminate the most common dose-limiting side effect of this class of drugs.

AMPK ACTIVATORS FOR THE TREATMENT AND PREVENTION OF CHRONIC POSTSURGICAL PAIN

Pain is an obvious consequence of surgery. While acute postsurgical pain can be controlled by existing analgesics, the incidence of chronic pain after surgery is surprisingly high with rates as high as 50% for certain surgical interventions (94). The causes of chronic postsurgical pain seem to involve the release of cytokines (e.g. IL6, (95, 96)) and growth factors (e.g. NGF, (97–99)) from the incision site. These factors, as in the scenario of neuropathic pain, promote changes in the phenotype and function of DRG neurons making these neurons hyperexcitable (100, 101). A variety of risk factors for chronic postsurgical pain have been identified and two of the most prominent are pain prior to surgery and/or a previous surgical intervention (94, 102). For instance, the rate of chronic pain after a revision to a knee replacement surgery is much higher than for the original knee replacement surgery suggesting that the establishment of pathological pain plasticity from the first surgery alters the response to the second stimulus (the revision surgery) (103). An interesting aspect of surgery is that the procedure causing the injury is planned so interventions aimed at preventing the development of chronic postsurgical pain can be put in place at the time of the plasticity-inducing event. Unfortunately, no current treatments have been shown to prevent the development of chronic pain after surgery and there are indications that the most common treatment for acute postsurgical pain after surgery, opioids, increases the probability of development of chronic postsurgical pain (104).

Factors thought to promote chronic pain after surgeries stimulate signaling events in nociceptors innervating the incision site. These factors signal largely via mTORC1 and MAPK pathways (e.g. NGF and IL6), therefore, AMPK activators are a logical therapeutic option to prevent the development of pathological plasticity after incision. This idea has been explored using resveratrol, which activates AMPK and decreases ERK and mTORC1 signaling in DRG and TG neurons (105). Resveratrol application at the time of incision in mice, or 24 hours following incision, effectively reduces mechanical hypersensitivity evoked by the incision injury. Incision to the mouse paw changes the nociceptive system such that a

subsequent sub-threshold stimulus after the incision causes a prolonged mechanical hypersensitivity. This sub-threshold stimulus has no effect in naïve mice and is capable of evoking a pain state in “primed” mice even weeks after the incisional injury has healed. Hence, this form of plasticity may model the “second hit” scenario outlined above wherein the incidence of chronic post-surgical pain is very high (103). Crucially, local resveratrol treatment at the time of the incision completely prevents this priming suggesting that AMPK activation may be used as a preventative treatment for chronic postsurgical pain (105). It remains to be seen if other AMPK activators also have this effect on chronic postsurgical pain, however, recent studies suggest that curcumin also inhibits incision-induced pain and prevents priming effects created by the incisional injury (106). While the mechanism of action of curcumin was not explored in this study, curcumin activates AMPK in many cell types and likewise attenuates MAPK and mTORC1 signaling (107, 108).

AMPK ACTIVATORS FOR THE TREATMENT OF INFLAMMATORY PAIN

A recent study examined the effect of the AMPK activators AICAR and metformin in models of inflammatory nociception in mice (109). Both of these compounds alleviated inflammation induced pain hypersensitivity and reduced the inflammatory response. AMPK activator treatment also reduced pain-associated activation of MAPKs in the spinal cord and the induction of c-fos expression in dorsal horn neurons. It is unclear whether these effects were caused by inhibition of pain-related signaling events in the PNS therefore reducing afferent input to the spinal cord or if these effects were mediated by a central action of AMPK activators. Importantly, this study also evaluated the contribution of AMPK to inflammatory pain using a genetic approach. Here α2 AMPK subunit knockout mice showed enhanced inflammatory hypernociception and a complete loss of the pain relieving effects of AMPK activators suggesting that the α2 subunit is the key regulatory factor for the antinociceptive effects of AMPK activators (109). Going further, these investigators also generated conditional knockout mice lacking the α2 subunit either specifically in sensory neurons or in macrophages and granulocytes. Both of these strains of mice showed enhanced inflammatory nociception suggesting that sensory afferents and immune cells both contribute to AMPK-mediated effects in the context of inflammatory pain. An interesting aside to these findings is the recent discovery that salicylates activate AMPK, albeit at high concentrations (110). Salicylates were the first widely used drugs for inflammatory pain and their efficacy has been widely attributed to their irreversible inhibition of cyclooxygenase (COX) enzymes. While this is undoubted a major mechanism of action for these drugs, some of their pain relieving properties may also be mediated by AMPK activation. Salicylates are no longer widely used due to their severe gastrointestinal side effects but selective AMPK activators may provide inflammatory pain relief without the well-known side effect of COX inhibitors.

AMPK ACTIVATORS FOR THE PREVENTION AND TREATMENT OF DIABETIC NEUROPATHY

For several decades metformin has been the first line medication used for type II diabetes. Left unmanaged, diabetes causes damage to many organ systems. A common comorbidity of

diabetes is peripheral neuropathy. Diabetic neuropathy usually manifests as tingling and numbness in the distal extremities sometimes accompanied by burning pain (111). Animal and human histochemical studies indicate that diabetes can cause dieback of PNS epidermal endings (112–115), although this may not be related to pain (115), and animal studies have shown the development of ectopic activity in DRG neurons (116, 117). Hence, while the cause is metabolic disease, diabetic neuropathy shares properties with trauma-induced neuropathic pain and CIPN.

An interesting question arising from the variety of pharmacological treatments used to control glucose levels in type II diabetics is whether any of these treatments are associated with higher levels of neuropathy and/or whether any of these treatments are neuroprotective. While no conclusive studies have been done in clinical populations, there are some indications that insulin treatment is associated with increased neuropathy incidence while metformin may protect against peripheral neuropathy. For instance, in a cohort of patients enrolled in a large study examining coronary artery disease and type II diabetes, insulin use was associated with a higher rate of neuropathy while metformin use was associated with a protective effect independently of type II diabetes duration, glycemic control and several other disease characteristics (118). A very recent study in rats adds important support to this clinical study. Here, a high fat diet (HFD) and streptozotocin (STZ) model of diabetes (HFD/STZ) was used to explore whether metformin alters the development of neuropathic pain independently of plasma glucose levels. These investigators found that while metformin treatment, started 4 days following the induction of diabetes, did not influence blood glucose levels over several weeks of treatment, metformin did completely block the development of mechanical hypersensitivity caused by the HFD/STZ treatment (119). Interestingly, this study did not find any indication of microglial or astrocyte activation in the spinal dorsal horn accompanying the development of neuropathic pain in these rats (119). Hence, it is unlikely that effects on the spinal immune response to nerve injury mediated the effects of metformin in this study because no such responses were observed. Future studies should examine the molecular mechanisms through which metformin, and potentially other AMPK activators, protect the PNS during metabolic disease. One possibility is through the induction of apolipoprotein E expression, a well-known neuroprotective factor, which is induced in the PNS by metformin treatment (120).

AMPK may play a mechanistic role in the development and maintenance of neuropathy induced by diabetes. Hyperglycemia alters the energetic profile of DRG neurons and their mitochondria and leads to a downregulation of AMPK expression, phosphorylation and activity (121). This decrease in AMPK appears to play a causative role in diabetic neuropathy as augmenting AMPK expression with viral vectors has positive effect on the energetic profile of DRG neurons and the AMPK positive modulator resveratrol has similar effects. Importantly, in vivo, resveratrol reverses established losses in thermal sensitivity and re-establishes epidermal innervation density in STZ-treated rodents (121). This study did not assess whether resveratrol had an impact on DRG neuron excitability or behavioral measurements of pain in these models. Nevertheless, these findings, combined with those discussed above, strongly implicate AMPK as a target for the treatment of multiple aspects of diabetic neuropathy.

METFORMIN AS A NOVEL PAIN THERAPEUTIC

The studies discussed above demonstrate a powerful effect of metformin in trauma-induced, CIPN and diabetic neuropathic pain models. Since metformin is one of the most widely prescribed drugs in the world, why have the pain-modifying effects of this drug not been evident amongst the population that is already taking this medication? One obvious reason is that metformin is unlikely to act as an analgesic. The drug does not produce acute analgesia in rodents and its antihyperalgesic effects take several days to emerge (41, 43). Additionally, multi-day administration is required to prevent the development of neuropathic pain from the onset of treatment in the case of CIPN or diabetes (92, 119). Here, again, it is important to recognize that the available data suggest that metformin prevents the development of diabetic neuropathy in patients and animal studies support this conclusion (118, 119). Therefore, a strong anti-neuropathic pain effect of metformin may exist in clinical populations but has gone unseen for decades due to the protective nature of the drug. Similar data is emerging in the cancer literature where metformin appears to have a preventative effect on certain types of cancers (93), although these cancers may have little relation to diabetes, unlike neuropathy. Another example comes from a retrospective study of low-back pain patients taking metformin for diabetes. In an age and sex matched cohort of patients with lumbar radiculopathy seeking care at a pain clinic, daily pain scores were lower in the metformin treated cohort than in the control cases (122). Clearly, prospective studies are needed to understand the effects of metformin in humans with neuropathic pain. However, the combination of these clinical findings with the emerging preclinical work is promising for the possibility that this safe, inexpensive therapeutic may have utility for the treatment of neuropathic pain. In addition, more preclinical work is needed to ascertain the mechanism of action of metformin in relation to its effects on the PNS. While the available data points to AMPK, metformin is neither potent nor specific as an AMPK activator so other mechanisms could be at play with this drug.

DEVELOPING NOVEL THERAPEUTICS FOR PAIN TARGETING AMPK

Above we have discussed the rationale for targeting AMPK for the alleviation of pain as well as the evidence indicating that this can be an effective target. Three pharmacological approaches have been used to target AMPK in these investigations: complex I inhibition (e.g. metformin), direct positive allosteric modulation of AMPK (e.g. A769662) and prodrugs that are converted to AMP-like molecules to mimic the endogenous orthosteric modulator of the kinase (e.g. AICAR). Which of these approaches is better for the modulation of pain? The simple answer is that insufficient data is available at this point to reach a conclusion (available data for these compounds and genetic approaches is summarized in Table 1). Having said that, there are good reasons to pursue each of these approaches, which will be discussed below.

Complex I inhibition is widely thought to be the mechanism of action of metformin (123), although other targets have been described recently, including inhibition of AMP deaminase (124). Therefore, based on the strong preclinical and emerging clinical evidence with this compound there are excellent reasons to pursue more potent and efficacious complex I inhibitors that activate AMPK via this mechanism. Recently, several molecules that fit this

description have been described (125, 126) but to date none of them have been tested in preclinical models or even on DRG or TG neurons. One potential reason to favor complex I inhibitors versus other mechanisms of action for regulating AMPK is the emerging evidence for mitochondrial dysfunction in several forms of neuropathic pain (91, 127). Metformin is known to induce mitophagy (128) and may play a role in clearance of damaged mitochondria from distal nerves in neuropathic pain states. Future studies should examine this potential molecular mechanism in more detail.

Another option is to create prodrugs that are converted into AMP mimetics in cells to orthosterically stimulate the kinase through binding to the AMP site on the γ subunit of the heterotrimeric kinase. This is the well-known mechanism through which AICAR (5-aminoimidazole-4-carboxamide (AICA) riboside) acts where the prodrug is taken up by cells and converted to ZMP (AICA ribotide) via a phosphorylation event (129, 130). ZMP then binds the AMP site on γ subunits of AMPK (131). AICAR has not been examined as widely as metformin or A769662 in DRG or TG neurons, however, it is efficacious in inflammatory pain models (109) and its action on neurons has been described in detail in other systems (132). One problem with AICAR is its clear lack potency, however, the compound is safe at relatively high concentrations in rodents and has been taken by humans, albeit for performance-enhancing uses in elite athletes (133). Safety in humans has never been evaluated. One potential advantage of the prodrug route of drug administration is that this mechanism does not require raising AMP levels in cells (131), as complex I inhibition does, and therefore may avoid adverse effects produced by alterations in cellular energy homeostasis. It may even be possible to engineer prodrugs that would be selectively converted into AMP binding mimetics only in cells expressing certain enzymes. To date this approach has not been explored.

A final option is through direct allosteric modulation of the heterotrimeric kinase. The first well-known example of such an allosteric AMPK activator is A769662 (134). This compound requires the presence of a β1 subunit to activate the kinase and appears to protect the kinase from downstream dephosphorylation therefore prolonging kinase activity (135). Other similar acting molecules have now been described but with different binding sites (136). The total number of potential allosteric binding sites on the kinase is not known and this number may vary depending on the heterotrimeric makeup of the complex in individual cell types. This creates some exciting opportunities for drug discovery if certain cell types of interest express distinct heterotrimeric complexes. Two α subunits, 2 β subunits and 3 γ subunits have been identified (131, 137). The only cell-type specific expression described is for the γ3 subunit that is exclusively expressed in skeletal muscle in rodents and humans (138). In the DRG and TG, β1 subunit expression is clearly functionally relevant due to the impressive effect of A769662 on signaling pathways in these cells (43). Gene expression atlases from rodent and human databanks (biogps.org) indicate that DRGs contain mRNA for α1, α2, β1, β2, γ1 and γ2 subunits. Future work will need to focus on cell-type specific expression of these subunits, likely using in situ hybridization, to determine if specific allosteric modulators can be created to target heterotrimeric complexes found in nociceptors versus other cell types.

While the potential for allosteric modulators with some cell-type specificity is an advantage of this approach, there are other clear benefits. The most obvious one is potency. While a few potent AMPK activators have been described for other mechanisms of action, all allosteric modulators described to date are at least mid μM level potency compounds (136, 139). Another is AMPK specificity; because these compounds bind to allosteric sites on the kinase, or its regulatory subunits, the potential for highly specific compounds is improved versus binding to adenine nucleotide sites that may be shared by many targets (131). Finally, this mechanism is not dependent on activation of upstream mechanisms (140), such as LKB1 activation (141), and protects the enzyme from deactivation by phosphatases (142). This also offers an increased level of specificity insofar as these upstream kinases may have other downstream targets other than AMPK leading to unwanted engagement of signaling pathways in cells.

A potential fourth way forward is taking advantage of multiple mechanisms for regulation of AMPK to achieve pharmacological synergism. Here the use of direct positive allosteric modulators is critical because these compound influence phosphorylation status of the kinase. Concomitant stimulation of upstream kinases with biguanides or other mechanisms leads to enhanced AMPK activation with subsequent protection from phosphorylation mediated by positive allosteric modulators. Such synergistic effects have been shown in the heart with biguanides (metformin and phenformin) and A769662 (143). Similar synergism has been observed in hepatic cells with AICAR and A769662 (144) suggesting that combined allosteric and orthosteric site modulators can lead to synergism at least in some cells while bypassing upstream kinase activation. Future studies in DRG or TG neurons should assess the possibility for pharmacological synergism on excitability or signaling in these cells to devise new pharmacological strategies that might offer specificity or improved potency and efficacy.

UNANSWERED QUESTIONS: HOW DOES AMPK ACTIVATION REGULATE/ REVERSE PAIN PLASTICITY?

AMPK activation negatively regulates a broad variety of signaling pathways involved in anabolic processes in cells. These include translation regulation pathways, lipid metabolism pathways and transcriptional regulation signaling events. However, these signaling pathways are not the exclusive downstream targets for AMPK as ion channel (145) and GPCR regulation in neurons has also emerged as an important target for AMPK. Major targets include potassium channels (146–148), sodium channels (149, 150) and $GABA_B$ GPCRs (151), however, other targets will undoubtedly be discovered as progress on the role of AMPK in the nervous system continues to progress.

In terms of how AMPK targets DRG neurons, 3 major mechanisms have been described: negative regulation of translation control pathways that increase DRG excitability, negative regulation of ion channel phosphorylation by MAPKs, likely targeting voltage gated sodium channels (points 1 and 2 reviewed in Fig 2A – C, (43, 105)) and positive regulation of dysfunctional mitochondrial metabolism (119). These studies were discussed in detail above but the mechanisms responsible for the disease modifying effects of AMPK activators in

neuropathic pain models and the prevention of chronic postsurgical pain have not been clearly elucidated. These downstream mechanisms are of clear interest as they would provide important insight into how these disease states progress, become chronic and might ultimately be reversed.

One possibility is that AMPK activation reverses pathological changes in gene expression induced by injury in the axonal compartment of DRG neurons. In support of this hypothesis is the evidence that injury to DRG neurons induces a profound reorganization of the translational machinery in axons including elongation factors, ribosomes, kinases involved in translation regulation and mRNAs and that AMPK activation normalizes increased translation in the sciatic nerve after injury (43). How can this be achieved? An obvious culprit is blockade of translation regulation pathways that leads to a reduction in the activity-dependent expression of genes in the axonal compartment that regulate excitability. However, this answer does not readily explain how disease modification is observed even after pharmacological stimulation of AMPK is removed (41). An alternative is that AMPK activation stimulates cellular events that permanently alter the capacity for changes in gene expression induced by injury. One such potential mechanism is the induction of P bodies by AMPK activators (152). P bodies, or processing bodies, are cellular organelles enriched in mRNA decapping enzymes where the life cycle of cytosolic mRNAs are terminated (153). AMPK activators robustly stimulate P body formation in DRG neurons and their axons *in vitro* (152). It remains to be seen if this mechanism is engaged *in vivo*. However, a scenario can be envisioned wherein P body induction in the axons of injured DRG neurons effectively rids the axonal compartment of mRNAs that are trafficked following injury thereby terminating altered gene expression even after cessation of pharmacological treatment (Fig 3).

This speculative line of reasoning places an important emphasis on the subclasses of mRNAs that are trafficked into the axonal compartment of DRG neurons under normal conditions and following injury. Overwhelming evidence indicates that injury to the axonal compartment of DRG neurons alters the trafficking of mRNAs (43, 85) and that only subsets of mRNAs are trafficked under basal conditions and after injury (31, 88) (Fig 3). While no specific mRNAs have been conclusively implicated in local translation and the regulation of neuropathic pain, a few targets have been identified with important implications. One of these is the mRNA for the tetrodotoxin-resistant voltage gated sodium channel specifically expressed in nociceptive DRG neurons, $Na_v1.8$ (86, 87). Importantly, Nav1.8 expression is altered by injury wherein expression in the cell body is decreased but expression at the site of injury is augmented (154). Since injury increases Nav1.8 mRNA localization to the axons of DRG neurons (86, 87) this localized change in expression may be mediated by local translation. Removal of Nav1.8 mRNA from the axonal compartment by AMPK activation and P body induction would be expected to remove a potential contributing factor to ectopic activity generation at the site of injury. Another target mRNA is that for the transcription factor CREB (59, 155). CREB axonal synthesis is induced in embryonic DRG axons by NGF treatment (155) and in the axons of DRG neurons from adults by IL6 treatment (59). This is linked to changes in plasticity on the level of transcription responsible for the persistence of hyperalgesic priming and therefore may be related to pathological pain plasticity (59). Persistent translation of axonal CREB may provide a consistent source of a

retrograde signal from injured nerves linking local injury to pathological changes in transcription that regulate excitability processes.

The examples cited above are but two of many possible factors that may act in concert to regulate complex disease states like neuropathic pain. Hence, future work should focus on gaining a better understanding of the repertoire of mRNAs that are found in the axonal compartment of adult DRG neurons and how these mRNAs may influence pathological plasticity. Systems biology approaches have shed light on what kinds of mRNAs localize to distal sites in neurons (dendrites or axons) with important implications for plasticity. First, these studies have made it clear that mRNA transport to distal sites in neurons is not simply a process of diffusion where mRNAs from diverse protein classes are found at these distal sites due to stochastic events. To the contrary, data from embryonic DRG neuron axons suggests that mRNAs encoding membrane-spanning proteins are completely excluded from the axonal compartment (156). It remains to be seen if this is the case in adult DRG neurons with or without injury but the finding has critical implications for the $Na_V1.8$ data discussed above. Distal sites in neurons are enriched in mRNAs encoding proteins: containing post-translational modification sites, such as phosphorylation sites; possessing high intrinsic disorder, e.g. proteins that can assume multiple conformations like kinases; and with short half-lives (157). This final attribute may be an important component for the localized regulation of signaling insofar as it would afford the termination of altered signaling when a stimulus is removed via degradation of nascently synthesized proteins. Hence, it appears that the subsets of mRNAs that localize to distal sites in neurons prime these cells for localized changes in signaling (157). This likely helps to explain why local translation has emerged as a key theme in synaptic plasticity in the CNS (24, 158–160) and may be linked to pathological plasticity in the PNS that underlies neuropathic pain (10, 13, 16, 21).

CONCLUDING REMARKS

Pathological pain represents a key medical problem that is inadequately treated by current therapeutics. Strong evidence links signaling events regulated by AMPK to pathological pain caused by nerve injury, inflammation and surgery. In line with these findings, pharmacological and genetic studies in rodents demonstrate that AMPK activation decreases behavioral signs of pathological pain, inhibits signaling associated with pathological pain and reduces DRG and TG neuron excitability. Several therapeutic approaches can be explored for exploiting this signaling pathway for the treatment of pain in humans including prospective trials for existing therapeutics and drug discovery efforts for novel therapeutics. Suggestions of disease modification in multiple preclinical pain models makes gaining a better understanding of downstream effectors of AMPK signaling in this context an important future research direction that may lead to further target identification. Therefore, the existing evidence supports increased efforts to understand the role of AMPK in pathological pain with an eye toward developing a new generation of pain therapeutics with disease modifying properties.

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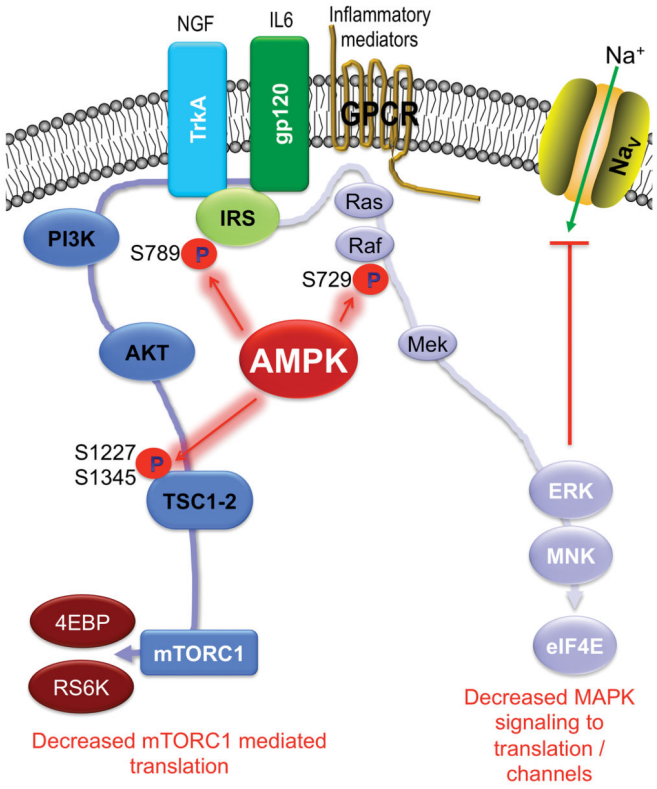

Figure 1. AMPK negative regulation of signaling pathways associated with pain plasticity

Trk receptors, such as the NGF receptor TrkA, the gp120 protein, which transduces IL6 signals and GPCRs responsible for detecting inflammatory mediators are capable of signaling via mTORC1 and MAPK pathways in nociceptive DRG and/or TG neurons. AMPK activation negatively regulates these signaling events via phosphorylation of a number of key signaling molecules. AMPK phosphorylates the Trk receptor signaling adaptor protein IRS at Ser789. This results in dampened signaling via Trks and may also negatively influence gp120 signaling. A key phosphorylation event for downstream regulation of mTORC1 signaling by AMPK is TSC1-2 phosphorylation at Ser 1227 and/or Ser 1345. Both of these phosphorylation events reduce signaling via the mTORC1 pathway in an AMPK activation-dependent fashion. Finally, B-raf phosphorylation at Ser 729 by AMPK decreases MAPK signaling since this small GTPase is a key signaling molecule linking receptors to MAPK signaling pathways. A potential consequence of AMPK activator-mediated decreases in MAPK signaling is a decrease in the phosphorylation of voltage-gated sodium channels such as $Na_v1.7$ which can lead to a reduction in excitability of DRG or TG neurons.

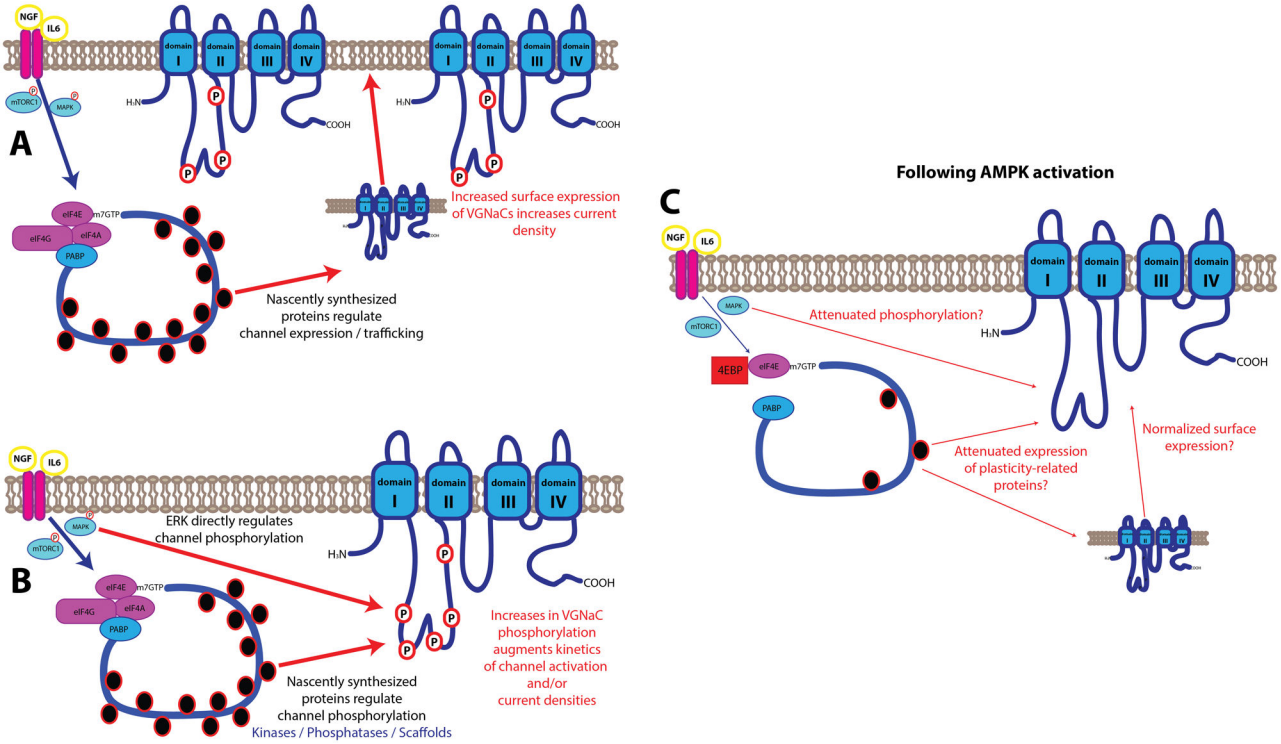

Figure 2. MAPK and mTORC1 signaling in regulation of neuronal excitability and the consequences of AMPK activation

A) Factors associated with pathological pain such as NGF and IL6 signal to the translation machinery via mTORC1 and MAPK and this is linked to increases in the excitability of DRG and TG neurons. One possible mechanism is that nascently synthesized proteins are involved in the trafficking of channels to the membrane of these neurons. This could lead to enhanced surface expression of these channels, for instance, increased trafficking of voltage gated sodium channels (VGNaCs). B) An alternative mechanism is that signaling via these pathways regulates factors associated with the phosphorylation of channels, thereby increasing DRG and TG neuron excitability. Here MAPK signaling itself can increase channel phosphorylation, including at VGNaCs, and nascently synthesized proteins may also play an important role in regulating channel phosphorylation either via the increased synthesis of kinases involved in these phosphorylation events or via enhanced expression of scaffolding proteins that facilitate channel phosphorylation. C) AMPK activation inhibits mTORC1 and MAPK signaling and would therefore be expected to reduce nascent synthesis of plasticity-related proteins and dampen phosphorylation events downstream of MAPK signaling. This results in decreased DRG and TG neuron excitability. While the precise mechanisms are not known, this is likely linked to channel trafficking or channel phosphorylation or both and VGNaCs may be primary targets for the positive actions of mTORC1 and MAPK signaling and their negative regulation by AMPK.

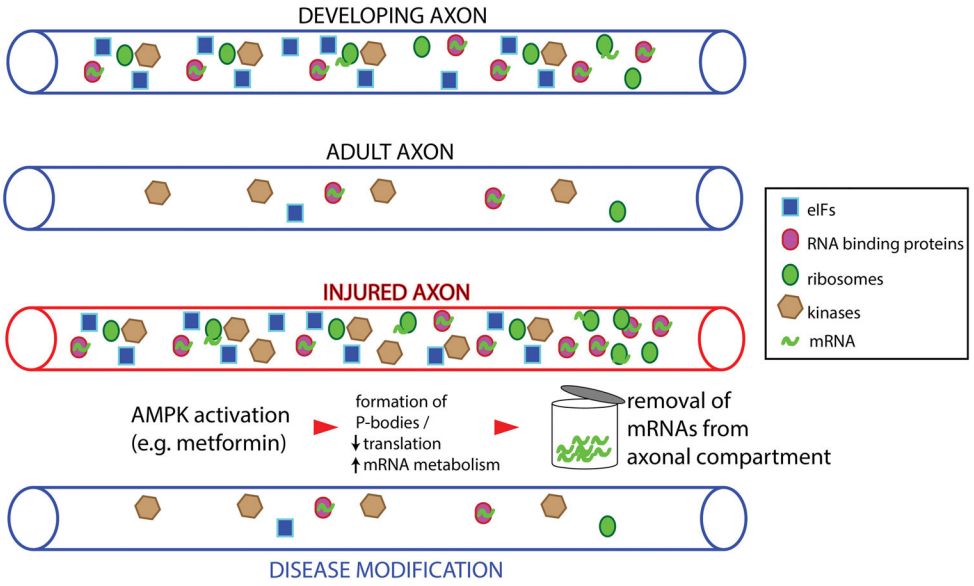

Figure 3. P body regulation by AMPK and its possible role in disease modification in neuropathic pain

Developing DRG axons contain translation regulation signaling components, RNA binding proteins, ribosomes and many mRNAs but adult axons are relatively depleted of many of these factors although they are still present at lower levels. Injury leading to neuropathic pain causes a reorganization of these factors potentially recapitulating a developmental program initiated by axonal injury. AMPK activators like metformin reverse neuropathic mechanical hypersensitivity and this neuropathic pain symptom does not return upon cessation of treatment suggesting disease modification. Metformin also normalizes increased protein translation in injured nerves. Recent findings indicate that AMPK activators, like metformin, robustly stimulate P body formation in DRG neurons and their axons. Since P bodies are a primary site of mRNA decapping and subsequent metabolism in cells, this suggests that AMPK activation may instigate a program wherein maladaptive changes in translation regulation signaling are reversed through the removal of mRNAs from the axonal compartment. We speculate that this mechanism could then effectively shut off a gene expression program that alters the excitability of these neurons leading to neuropathic pain disease modification.

Table 1

Summary of pharmacological and knockout mouse findings on DRG and TG neurons and/or in preclinical pain models.

			BEHAVIORAL MODELS				
AMPK activator	**DRG/TG neuron excitability**	**DRG/TG neuron signaling**	**Trauma-induced neuropathic**	**Chemotherapy-induced neuropathic**	**Diabetic neuropathy**	**Zymosan inflammation**	**Formalin test**
Metformin	↓ramp spiking	↑AMPK ↓mTORC1	↓mechanical	↓mechanical	↓mechanical	↓mechanical	↓phase II
AICAR		↑AMPK ↓mTORC1 ↓MAPK				↓mechanical	↓phase II
Resveratrol		↑AMPK ↓mTORC1 ↓MAPK	↓mechanical (incision model)		↓peripheral neuropathy		
A769662	↓ramp spiking	↑AMPK ↓mTORC1 ↓MAPK	↓mechanical				
α2 global knockout mice						↑mechanical	↑phase II
α2 sensory neuron specific knockout						↑mechanical	↑phase II
α2 macrophage and granulocyte knockout						↑mechanical	↑phase II

Data discussed in the text is summarized by AMPK activating compound or genetic manipulation.

Blank cells indicate that testing has not been conducted.