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REVIEW ARTICLE

The Proteomics of Intrathecal Analgesic agents for Chronic Pain

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Abstract: Chronic pain remains a challenging clinical problem with a growing socio-economic burden for the state. Its prevalence is high and many of the patients are of work age. Our knowledge regarding the pathophysiology of chronic pain is poor. The consensus view is that the central nervous system plays a key role in the persistence of pain after an initiating event has long ceased. However the specifics of this biological response to an initiating event remains unclear. There is a growing body of evidence to support the concept that a central neuroimmune response is initiated and a number of small peptides have been implicated in this process following cerebrospinal fluid analysis in patients with chronic pain. This central biosynthetic peptide response leads to a process called central sensitization. Therapy is aimed at modulating and even inhibiting this response. However current pharmacological therapeutic options are limited in efficacy with significant deleterious side effect profiles. Proteomic studies extend single molecule analysis by identifying the components of biological networks and pathways and defining their interactions. This tool offers the potential to provide a molecular overview of the biological processes involved in chronic pain. It will also facilitate examination of gene-drug interactions. This technique offers a mechanism of defining the central biological responses that result in chronic pain and this information may facilitate the development of better therapies.

Keywords: Analgesia, intrathecal, proteome, pain.

1. INTRODUCTION

Proteomics describes the scientific pursuit of the entire complement of proteins in a specific tissue sample. This collection of proteins is referred to as the proteome. While it is rare to catalogue the complete proteome, evolving technologies have significantly improved the rate of accurate protein identification. Proteomics differs from genomics which is the study of an organism's gene expression profile. Proteomic technology provides more valuable information because mature proteins determine the biological activity of an organism.

Proteomic technology has advanced significantly over the past decade. It is now possible to catalogue an organisms entire proteome [1]. Identifying the proteome of clinically relevant tissues has become the goal of researchers in many disease states [2]. Proteomic techniques can elucidate translational and post-translational modifications in the nervous system associated with persistent pain. Neuropathic pain is a disease that has a significant socioeconomic impact, and is challenging to manage with limited therapeutic options. Pain researchers have utilized unbiased protein analysis techniques to identify key proteins in the generation and maintenance of neuropathic pain [3]. Identification of these proteins, and their role, may reveal novel therapeutic targets or useful biomarkers. Furthermore, pain researchers have documented alterations in these key "pain-related" neuropeptides following interventions in humans [4]. This is a key step in translating animal data to the clinical setting.

Nociceptor activation can result in both prolonged and enhanced synaptic activity in central nociceptive connections leading to central sensitization [5]. As regularization of this central neuronal response is a target of therapy, the intrathecal route of analgesic agent delivery seems attractive.

The intrathecal route offers direct drug delivery to the central nervous system. This offers the avoidance of bioavailability issues of alternative systemic delivery routes. On the other hand "one shot" techniques are of little value in chronic disease so a catheter must be sited for continuous delivery. While an externalized system may be used in the management of end of life care for predicted short term opioid delivery, a programmable implanted system is the only viable option for the management of chronic pain. An intrathecal drug delivery system (IDDS) usually consists of a programmable pump implanted subcutaneously in the abdomen and connected to the cerebrospinal fluid *via* a tunneled intrathecal catheter. Anaesthetists administer a number of agents intrathecally, including opioids, neostigmine, ketorolac and dexmedetomidine in conjunction with local anaesthetic agents (primarily bupivacaine and ropivicaine), in the management of acute postoperative pain [6].

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In 2012, the Polyanalgesic Consensus Conference (PACC) produced guidelines on the management of chronic pain with intrathecal drug deliver systems [7]. Morphine, fentanyl and hydromorphone are the most commonly used opioids. Intrathecal bupivacaine is used predominantly in the management of cancer pain in combination with opioids. Intrathecal ziconotide and opioids may be used for refractory neuropathic pain [8]. Intrathecal steroid therapy has been used to relieve intractable postherpetic neuralgia [9]. Intrathecal baclofen is very successfully used in the management of spasticity [10]. Other drugs used less frequently include clonidine and sufentanil. Patients considered for these interventions are generally complex cases, and a detailed management plan is required before initiation of intrathecal therapy to identify patients most suitable and avoid treatment failure, adverse effects and mortality [11].

Whilst morphine is the most commonly used intrathecal analgesic, its effectiveness in the longer term has been called into question [12, 13]. Data on the mechanism of action and efficacy of the commonly used intrathecal agents is sparse, and the cost of treatment is significant. Proteomic analyses of central nervous tissue in pain models, and following intrathecal drug deliver, may identify novel therapeutic targets or biomarkers, and clarify the mechanism of action of intrathecal agents. This article will examine the current knowledge regarding the proteomic changes associated with intrathecal morphine, hydromorphone, fentanyl, bupivacaine, methyprednisolone, baclofen, clonidine, and ziconotide. To our knowledge, this is the first literature review addressing proteomic changes associated with intrathecal agents.

2. PROTEOMIC ANALYSIS

Fig. **1** represents the techniques commonly used in proteomic analysis. The clinically relevant tissue is prepared for processing. The tissue is lysed and homogenised, and the lysate is centrifuged. The supernatant is removed for proteomic analysis. Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) is used to separate different proteins by isoelectric point, molecular weight, solubility, and relative abundance [3]. It's possible to separate thousands of proteins into individual "gel spots". These spots may be visualised with chemical stains or fluorescent markers. Quantification of the individual proteins is achieved by calculating the intensity of the staining.

A limitation of 2D-PAGE is it is failure to separate less common, large, hydrophobic molecules (*e.g.* membrane receptor proteins). Early studies failed to identify these proteins, and many other protein spots were labeled as "unknown" [14, 15]. More recent proteomic studies have used liquid chromatography to improve protein separation [16].

Identification of the protein requires mass spectrometry (MS) - (matrix-assisted laser desorption ionisation time-of-flight [MALDI-TOF] or surface-enhanced laser desorption

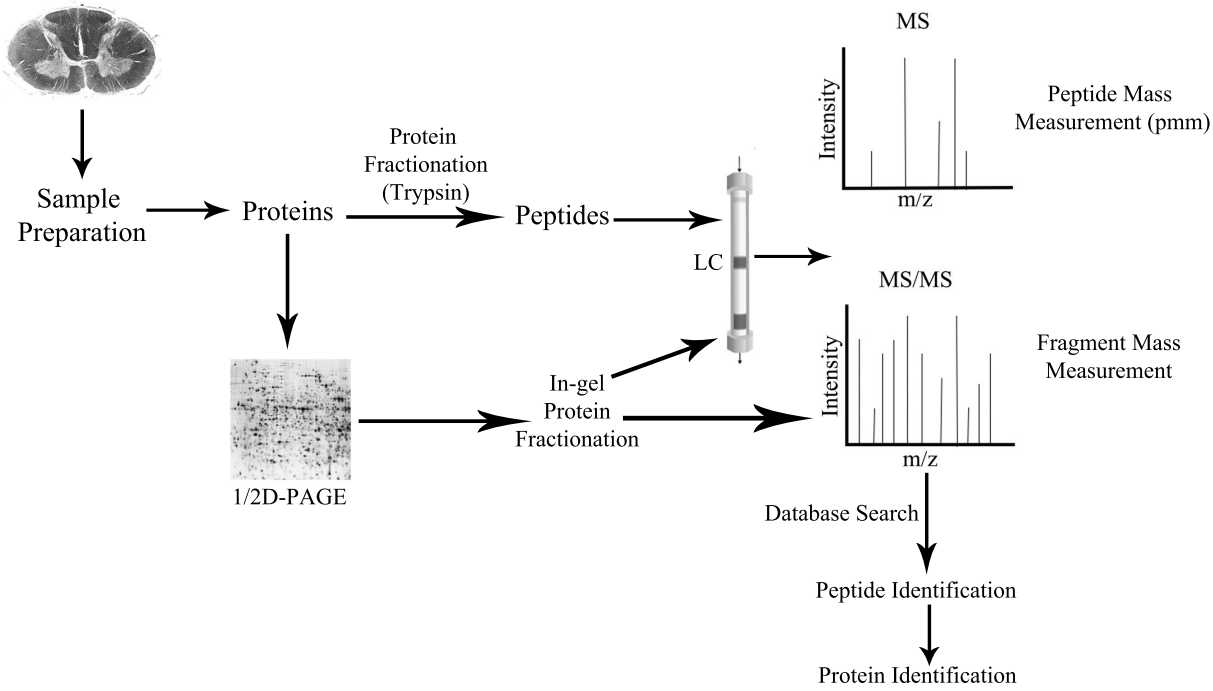

Fig. (1). Schematic of Proteomic Analysis. The tissue for analysis is prepared to isolate the protein complement. The proteins may be fractionated to peptides by a sequence-specific enzyme like trypsin. The peptides are separated by liquid chromatography (LC). They are then analysed in a mass spectrometer (MS). The MS identifies the mass of the sample peptides, fragments the peptides, and measures the mass of the peptide fragments. This is called tandem mass spectrometry (MS/MS). This data is entered into a database, and the peptides and original proteins are identified. Proteins may also be separated by a one or two-dimensional polyacrylamide gel electrophoresis (1/2D-PAGE). The resulting gel spots may represent discrete proteins, and following in-gel trypsin fractionation and/or LC, the peptides and proteins are identified with MS/MS.

ionisation time-of-flight mass spectrometry [SELDI-TOF]). The protein spots are resected from the gels and the proteins are digested to peptides by trypsin. The MS performs peptide mass matching (pmm). A number of databases exist that allow identification of these peptides and ultimately identification of the original proteins [17].

More recently, multidimensional protein identification technology (MudPIT) has enhanced data acquisition in proteomic analysis [18]. MudPIT uses non-gel techniques for peptide separation. Two-dimensional liquid chromotography (LC) facilitates peptide separation, and peptide identification is achieved with tandem MS (MS/MS). MudPIT is a more sensitive protein identification technology.

Proteomic technology allows identification of a peptide or protein, but quantification is also possible [19]. Quantification in proteomics is achieved by one of the two methods - the stable isotope-based method and the label-free method. The isotope-based method involves the addition of a "heavy" molecule into the peptide, either by methylation by a chemical reagent or by culturing a cell in an isotope rich medium. The isotope-labelled peptide is mixed in with normal peptides. The MS can determine the ratio of labelled versus normal peptides, and estimate from this the absolute amount of a protein in a sample. The label-free method involves estimating the absolute amount of protein from the number of peptide fragmentation events during MS. Quantification techniques have not been employed in the studies reviewed in this article. However, in future proteomic studies of therapeutic interventions quantification techniques will allow researchers to quantify the magnitude of the therapeutic effect.

3. CNS PROTEOME IN PAIN MODELS

Most of the relevant proteomic studies used a rat model of neuropathic pain (spinal nerve injury), and tissue from the lumbar enlargement segments were compared to controls to gather information on the CNS proteomic changes associated with neuropathic pain.

An early proteomic study associated decreased levels of creatine kinase B with neuropathic pain in a nerve injury model [20]. Zou *et al.* documented the role of Protein Kinase C (PKC) gamma in the regulation of neuropathic pain using proteomic methods [21]. Activation of PKC gamma in the central nervous system is associated with neuropathic pain. Similar to the other PKC family members, PKC gamma is an important second messenger, and is involved in many cellular pathways. Zou *et al.* silenced the PKC gamma gene in half of the rats with a stable RNA interference (RNAi). The pain behaviour of the rats improved in the group with downregulation of PKC gamma. In addition proteomic analysis allowed the group to correlate the improved pain with a variety of differentially expressed proteins. These proteins may occupy pathways that are regulated by PKC gamma and may be key to the development of neuropathic pain. Proteomic analysis of the synaptosome of the dorsal horn, during the maintenance phase of neuropathic pain, identified changes in protein expression and distribution within the dorsal horn cells [22].

Proteins identified in the above studies play roles in transmission and modulation of noxious information, plasma membrane receptor trafficking, cellular metabolism, heat shock proteins and chaperones, antioxidant proteins, cytoskeleton proteins, and apoptosis. They may be significant in the pathogenesis of neuropathic pain. Glutamate dehydrogenase 1 is upregulated following nerve injury, and glutamate receptor activation plays a key role in the development and maintenance of neuropathic pain. Metabolic enzymes like L-Lactate dehydrogenase, fumarylacetoacetase, gamma-enolase, and creatine kinase are upregulated. Transitional endoplasmic reticulum ATPase (TERA) and synaptosomal-associated protein (SNAP) are upregulated in neuropathic pain models, and are important proteins in plasma membrane receptor trafficking. Tubulin beta chain is a cytoskeleton protein that is upregulated in neuropathic pain models. Neuromas in animal models display similarly altered protein expression, in particular, structural proteins involved in cytoskeletal organisation (tubulin beta), metabolic enzymes (gamma-enolase and creatine kinase B), redox regulation (peroxiredoxin), cell signalling (annexin), protein folding or chaperone function (protein disulfide isomerase) [23]. Melemedjian *et al.* identified upregulation of Apolipoprotein E (ApoE) in a spinal nerve ligation model; ApoE is involved in nerve regeneration and repair following injury [24].

Nerve tissue is often required in the proteomic analysis of neuropathic pain. This normally limits researchers to animal experiments. However, Oki *et al.* performed a proteomic study of resected nerves in CRPS patients [25]. This study linked a deficiency of methallothionein in injured nerves with the syndrome. Methallothionein protects against nerve injury, and promotes regeneration.

4. INTRATHECAL (IT) AGENTS

4.1. Opioids

The primary target for IT opioids are the opioid receptors in the dorsal horn of the spinal cord. It has been suggested that mortality in patients receiving opioid therapy may be up to 3.89% at one year [26]. The data presented by Coffey and colleagues should be interpreted with caution as this refers to a cohort with several co-morbidities and in several cases a high initial IT dose was administered which may have contributed to the increased mortality observed. There was no data to suggest that pump malfunction contributed to the mortality statistics. Granuloma formation is associated with IDDS, but rarely presents with symptoms of spinal cord impingement [27]. Other complications include dysfunction of the pump hardware, infections, CSF leak, and side effects of the medication.

While morphine is the only IT opioid with Food and Drug Administration (FDA) approval for the management of chronic pain, other opioids like fentanyl, sufentanil, methadone, meperidine, hydromorphone and tramadol have also been used in IDDS. Morphine, hydromorphone, and fentanyl are recommended as first line IT therapy in nociceptive pain by the 2012 PACC [7].

4.1.1. Interaction of Morphine and the Proteome

As our knowledge of the CNS proteome in neuropathic pain evolves, we can focus our attention on the proteomic changes following therapy. If these key "pain-related" proteins are upregulated or downregulated in neuropathic pain, and they do actually play a role in this disease process, successful therapies should target similar proteomic networks.

The analgesic agent most commonly investigated in proteomic studies is morphine. Most studies are concerned with tolerance and addiction, and the role certain proteins play in these neurobiological processes. However, they give us an insight into the proteomic impact of this powerful analgesic.

A meta-analysis of 15 proteomic studies documented alterations in the proteome associated with morphine administration [28]. The studies in this meta-analysis involved rat, mouse, and human tissue from various different CNS sites. In order to perform a meta-analysis on such diverse data, the authors assigned a rat protein identification (if possible) to all differentially expressed proteins in these studies. They then performed statistical analysis of the data to identify proteins that were over-represented or under-represented across the 15 studies. This meta-analysis revealed that proteins associated with cell structure and intracellular protein traffic are upregulated, and proteins involved in carbohydrate metabolism are downregulated following morphine administration.

The impact of morphine on the proteome of non-human primates revealed a decreased abundance of proteins involved in the functional categories of energy metabolism, signaling, and maintenance of cell structure in the lymph nodes [29].

4.1.2. Intrathecal Morphine and the CNS Proteome

To date there is a sparsity of proteomic data regarding the impact of analgesics on animal pain. Four animal studies looking at the proteomic basis of morphine tolerance at the spinal cord level give us some insight into the impact of intrathecal morphine on the CNS proteome.

Shui *et al.* examined the spinal cord proteome following chronic intrathecal morphine exposure to identify the changes in protein expression [30]. Twelve male Sprague-Dawley rats had subarachnoid catheters inserted, and after one week 6 rats received 20 mcg morphine intrathecally twice daily and 6 rats received saline (control group) for 4 days. On day 5, both groups received morphine 20 mcg to assess analgesic potency. Noxious radiant heat was used to assess paw withdrawal latency (PWL). Analysis of lumbar enlargement segment tissue was performed with 2-DE and MALDI-TOF MS, and protein identification with Uniprot database. Eight proteins showed a significant difference in expression following chronic morphine exposure. Glutamate receptor interacting protein, Dynamin-II, Ras-related protein Rab-11, and Neuronal protein 22 were all upregulated. Centaurin alpha, Heat shock protein 70, Aconitase, and 3-Mercaptopyruvate sulfurtransferase were down regulated.

Liaw *et al.* studied the effect of intrathecal morphine on protein phosphorylation [31]. The authors hypothesised that specific phosphorylated proteins were involved in the development of morphine tolerance. They used the same methodology as previously described [30]. The results showed that in the 6 morphine-treated rats, 4 proteins were hypophosphorylated (glial fibrillary acidic protein, alpha-internexin, prohibitin, collectin sub-family member 10) and 6 were hyperphosphorylated (heat shock 70 kDa protein 5, 14-3-3 protein gamma, 14-3-3 protein zeta/ delta, tyrosyl-tRNAsynthetase, gamma-enolase, fructose-bisphosphatealdolase C).

Song *et al.* performed a study to investigate the role protein kinase C (PKC) gamma isoform plays in morphine tolerance [32]. Protein expression in the lumbar spinal cord of morphine tolerant rats, with and without PKC gamma-knockdown (RNAi-mediated gene silencing technique), was identified with 2-DE and MALDI-TOF. Paw withdrawal latencies were assessed with radiant heat to confirm morphine tolerance. Rats with PKC gamma-knockdown showed reversal of morphine tolerance indicating a possible role of PKC gamma in this condition.

Wen *et al.* performed a proteomic analysis of rat spinal cords associated with morphine tolerance and investigated the reversal of morphine tolerance with a NMDA receptor antagonist (MK-801) [33]. The male wister rats were implanted with an intrathecal catheter. Following a 4-day rest period, 4 different intrathecal infusion regimens were started - morphine 15mcg/hour (n=6), morphine 15mcg/hour and MK-801 5mcg/hour (n=6), and saline or MK801 infusion as the control groups. The morphine infusion group displayed upregualtion of protein glial fibrillary acidic protein (GFAP). This was partly reversed by the addition of MK 801.

4.1.3. Fentanyl

Fentanyl is considered a first-line IT therapy in nociceptive pain, and third line in neuropathic pain, by the PACC 2012 [7]. Intrathecal fentanyl in a formalin-induced pain model in a rat was associated with suppression of c-Fos expression in the spinal cord [34]. Noxious stimuli are associated with upregualtion of c-Fos [35]. The reversal of this process by IT fentanyl may support its use as an analgesic. However, no data on the interaction between IT fentanyl and the CNS proteome was identified for this review.

4.1.4. Hydromorphone

The PACC recommendation of IT hydromorphone is largely based on consensus, and limited evidence. Similar to morphine, it is a hydrophilic opioid, and demonstrates deeper penetration and greater bioavailability at the level of the dorsal horn of the spinal cord than more hydrophobic opioids like fentanyl [36]. We did not identify data on hydro-morphones impact on the CNS proteome in the literature.

4.2. Methylprednisolone

Methylprednisolone has been used in the treatment of neuropathic pain. It may be injected into the epidural or intrathecal space. A RCT by Kotani *et al.* suggested IT methylprednisolone was an effective therapy in the management of intractable neuropathic pain associated with

post herpetic neuralgia (PHN) [9]. However, more recent results do not support its use in PHN, and the well documented neurological side-effects of IT methylprednisolone have resulted in a decline in its clinical use [37]. IT methylprednisolone is associated with reduced expression of glial fibrillary acidic protein (GFAP) in a spinal nerve ligation model [38]. This demonstrates inhibition of glial cell activation in the development of chronic neuropathic pain.

One human study examined the impact of intrathecal methylprednisolone on the CSF proteome in patients with PHN. Lu *et al.* analysed the CSF proteome before and after treatment with methylprednisolone and lidocaine in eight patients with PHN. Patients received intrathecal injections of a mixture of 1.5 mL of 60mg methylprednisolone, 0.9 mL of 10% lidocaine, and 0.6 mL of 10% dextrose solution every week for 4 weeks. The pre-treatment and post-treatment CSF samples were analysed with 2-DE and MALDI-TOF, and proteins were identified with the MASCOT database. The proteins up regulated in the CSF post treatment were Gelsolin isoform alpha, Transferin, Haptoglobin, Apolipoprotein A-IV, and complement component 4A. The proteins down regulated following treatment were Nr-CAM protein Antithrombin III, alpha 1-antitrypsin, PRO2619, Angiotensinogen, Vitamin D binding protein, PEDF, Apolipoprotein E, L-PGDS. No correlation was found between pain relief and changes in protein expression.

4.3. Bupivacaine

Local anaesthetics disrupt signal transmission in neural cells by blocking voltage-gated sodium channels. They are often co-administered with other IT agents in chronic pain patients. This practice may decrease the rate of opioid dose escalation [39-41]. Bupivacaine is the only local anaesthetic included in the PACC treatment algorithms. It may be used as a second line agent in combination with opioids for the management of neuropathic and nociceptive pain [7]. We did not identify a proteomic analysis of IT local anaesthetic therapy.

4.4. Clonidine

Clonidine is an alpha-2 adrenergic agonist, that may be administered intrathecally in the management of acute and chronic pain. It may attenuate the neuroimmune activation associated with neuropathic pain. IT clonidine in rats with partial sciatic nerve ligation, resulted in reduced expression of NF-kappaB and p38 - markers of glial activation [42]. This therapy also inhibits the expression of pNR1 (a phosphorylated NMDA subunit) [43, 44]. IT clonidine can also reduce the release of excitatory neurotransmitters in the spinal cord of rats injected with capsaicin [45]. Inhibition of these key steps in central sensitisation may account for its anti-allodynic and anti-hyperalgesic effects. We did not identify a proteomic analysis of IT clonidine therapy.

4.5. Baclofen

Baclofen is a GABA-B receptor agonist. It has FDA approval as an IT therapy for spasticity. It may significantly improve symptom control and the quality of life in patients suffering from spasticity [10]. It may also be used as a fifth line therapy for neuropathic pain [7]. IT baclofen therapy in rat neuropathic pain models leads to reduced expression of spinal NR2B, an NMDA receptor subunit and phosphorylated cAMP response element-binding (CREB) protein [46, 47]. The therapeutic action of baclofen in neuropathic pain may be partly explained by the down-regulation of these proteins. We did not find data on the proteomic analyses of IT baclofen therapy.

4.6. Ziconotide

Ziconotide is a selective antagonist of the N-type calcium channel, and it controls neurotransmitter release [48]. It has FDA approval as an IT therapy in chronic pain patients. It is a first line therapy for neuropathic and nociceptive pain [7]. It is known to inhibit the release of glutamate, calcitonin gene-related peptide, and substance P in the dorsal horn of the spinal cord [49, 50]. We did not identify a proteomic analysis of IT ziconotide therapy.

5. DISCUSSION

Proteomics provides us with a global and unbiased snapshot of the proteome under investigation. We can document alterations in the proteome in response to a disease (neuropathic pain model) or a drug (intrathecal analgesia). Our understanding of the neuroproteomic changes associated with neuropathic pain have improved considerably in the last decade. However, our knowledge of the neuroproteomic effects of our more common intrathecal analgesics has lagged behind.

During our literature search, we did not find an animal neuropathic pain model that documented the neuroproteomic changes associated with intrathecal therapy. However, from the above data, we can identify common proteomic pathways that are associated with the initiation and maintenance of neuropathic pain, and the mechanism of action of intrathecal analgesics. This strategy could identify novel drug targets in the management of chronic pain [51].

5.1. Proteins Involved in Transmission and Modulation of Noxious Information in Dorsal Horn of Spinal Cord

The activation of glutamate receptors (AMPA, kainate, and NMDA) at the spinal cord level plays a key role in the initiation and maintenance of neuropathic pain [52]. Proteomic analysis in neuropathic pain models reveal an up-regulation of glutamate dehydrogenase 1 (GDH) which is a key enzyme in the regulation of glutamate metabolism [21, 22].

The glutamate-receptor interacting protein (GRIP) plays a role in targeting and localising the AMPA receptor at excitatory synapses [53]. GRIP may also have a function at inhibitory synapses. Intrathecal morphine therapy is associated with up-regulation of GRIP in the spinal cord [30].

The 14-3-3 protein family regulate a variety of cellular signaling pathways [54]. Some members of this family are up-regulated in neuropathic pain models [16]. They are also hyperphosphorylated following intrathecal morphine therapy [31].

5.2. Proteins Involved in Cellular Homeostasis and Metabolism

Cellular metabolism in the dorsal horn cells is increased in models of neuropathic pain [55, 56]. Enzymes involved in glycolysis show altered expression profiles at the spinal cord level following nerve injury. Gamma-enolase is up-regulated following spinal nerve injury [22]. It is also up-regulated in hyper-excitable neuromas in rodent saphenous nerves [23]. Fructose-bisphosphate aldolase C is downregulated in PKC gamma knockdown rats [21]. Intrathecal morphine therapy also alters the expression of these enzymes. Gamma-enolase, and fructose-bisphophate aldolase C are both hyper-phosphorylated in rats receiving intrathecal morphine [31].

5.3. Antioxidant Proteins and Heat Shock Proteins

Heat Shock Protein 70 (HSP 70) is up-regulated in a rat model of neuropathic pain. HSPs are up-regulated in the nervous system in response to stress or injury, and HSP 70 protects against oxidative damage [57]. In morphine tolerant rats, after intrathecal morphine therapy, HSP 70 is down regulated [30] and hyperphosphorylated [31]. Morphine therapy may negate the protective effects of HSP 70, and allow the apoptosis of dysfunctional neurons in neuropathic pain states.

Intrathecal methyprednisolone in humans with post-herpetic neuralgia is associated with a reduction in CSF lipocaline-type prostaglandin D synthase (L-PGDS) [58]. This enzyme catalyses the production of prostaglandin D_2 which is associated with centrally mediated nociception [59]. In addition, there is a reduction in CSF Apolipoprotein E (ApoE) following treatment. ApoE has potent anti-inflammatory and neuroregeneration properties [24]. It is up-regulated in the setting of nerve injury, and neuropathic pain.

5.4. Cytoskeleton Proteins

Neuronal degeneration is commonly associated with increased expression of neurofilaments. Alpha-internexin is a type-IV filament protein that is up-regulated in the dorsal horn following SNI. It is a neuron-specific cytoskeleton protein, and plays a role in neuroplasticity by facilitating axonal neurite elongation [60, 61]. The same neurofilament is hypophosphorylated in rats receiving intrathecal morphine [31].

Glial fibrillary acidic protein (GFAP) is an astrocyte-specific neurofilament that is overexpressed in the spinal cord of SNL rats [16]. GFAP is down regulated [33] and hypophosphorylated [31] in rats receiving intrathecal morphine. Nervous system plasticity depends on phosphorylation of key cytoskeletal proteins, and the hypophosphorylation of GFAP associated with morphine may play a role in opioid tolerance.

We must recognise that the above associations may simply be coincidence. There was no standardisation in the methodology of the studies. Depending on the experiment, there was significant variability in protein expression which may be attributable to different nerve injury models, tissue preparation and analysis. The intrathecal morphine studies were primarily focused on establishing the neuroproteomic profile of morphine tolerance.

We must also be aware of the limitations of the proteomic techniques employed in these studies. Protein analysis is substrate limited, and less-abundant, large, hydrophobic proteins (transmembrane proteins) were traditionally missed in earlier studies. Also proteins undergo post-translational modifications (PTMs) such as phosphorylation. These PTMs can impact on a protein's function, and are clinically relevant. One protein may undergo a number of different PTMs, and because they have a similar mass, MS may be unable to distinguish between them. PTMs may require special analytical processing to be captured and standard techniques may be inadequate. Enrichment strategies and advanced MS algorithms are improving the identification of PTMs. Proteomic experiments are particularly vulnerable to contamination from environmental proteins. They also incur significant time and resource costs. With more advanced techniques like MUDPIT (multidimensional protein identification technology), which eliminates gel electrophoresis and instead uses 2-dimensional liquid chromatography and tandem MS (MS/MS), we can create a more complete and reliable proteomic profile [62].

CONCLUSION

Our review identifies protein networks that are common to neuropathic pain in particular, and the mechanism of action of intrathecal analgesic agents. Proteins involved in transmission and modulation of noxious information, cellular homeostasis and metabolism, antioxidant proteins and heat shock proteins, and cytoskeleton protein may provide a common link. Future proteomic studies of neuropathic pain models should include a treatment group to correlate analgesia with protein expression. Standardisation of methodologies would facilitate more accurate interpretation of data.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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