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Role of Tat-Mediated PDZ Peptide Delivery in Pain Therapy

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ABSTRACT

Delivery of therapeutic peptides or proteins into tissues is severely limited by the size and biochemical properties of the molecules. Protein transduction domain (PTD)-mediated cargo transduction represents a novel and promising strategy to deliver biologically active peptides in vivo. The first PTD was identified from the HIV-1 transactivating transcriptional activator protein Tat in 1988. Since then, other PTDs have also been identified, including the third α-helix of the antennapedia homeotic transcription factor and synthetic peptide carriers. However, Tat PTD (amino acids 47 - 57) has shown markedly better ability for intracellular delivery than other PTDs. It has been demonstrated that fusion peptides containing the Tat PTD enter the central nervous system after systemic administration. Our previous study has shown that i.p. injected Tat-PSD-95 PDZ2 expresses in the central nervous system and significantly disrupts PDZ domain-mediated protein interactions between PSD-95 and N-methyl-D-aspartate receptor subunit NR2A/2B, thereby alleviating chronic pain. Therefore, Tat-mediated intracellular delivery can be used for systemic administration of analgesics in pain management.

Keywords*: *Tat Peptides*, *Protein Transduction Domain*, *Protein Interactions*, *PDZ Domains*, *Chronic Pain

1. Introduction

The discovery of small cationic peptides (8 - 16 amino acids in length) termed protein transduction domains (PTDs) or cell-penetrating peptides [1,2], which cross biological membranes, has emerged as a venerable Trojan horse to transport large, biologically active molecules, such as peptides, proteins, and oligonucleotides, into mammalian cells *in vitro*, as well as in preclinical models and clinical trials *in vivo*. Protein transduction was originally observed in 1988 after full-length HIV-1 transactivating transcriptional activator protein (Tat) was shown to enter mammalian cells, leading to transcriptional activation from an HIV-1 long-terminal repeat promoter construct [3,4]. Since the initial discovery of Tat-mediated transduction, other novel transduction domains have been identified within several other proteins, including the third α-helix of the antennapedia homeotic transcription factor [5-7] and synthetic peptide carriers, such as polylysine and polyarginine [8-10].

Chronic pain affects more than 50 million Americans per year and costs more than $100 billion each year in health care and lost productivity. It is often poorly managed by current drugs, such as opioids and non-steroidal anti-inflammatory drugs. Considerable evidence indicates that the development of central hyperexcitability and persistent pain involves the activation of *N*-methyl-D-aspartate receptors (NMDARs), which play an important role in the processing of nociceptive information [11-14]. However, directly blocking the function of NMDARs is therapeutically impractical because doing so would also impede other vital synaptic transmissions in the central nervous system (CNS). Postsynaptic density protein-95 (PSD-95), a PDZ-containing scaffolding protein, has been identified to interact and attach NMDARs to internal signaling molecules at neuronal synapses of the CNS [15,16]. This function suggests that PSD-95 might be involved in physiological and pathophysiological actions triggered via the activation of NMDARs in the CNS. Therefore, targeting PSD-95 protein represents a potential therapeutic approach for diseases that involve NMDAR signaling. NMDAR-PSD-95 protein interactions are mediated by a PDZ domain (a term derived from the names of the first three proteins identified to

contain the domain: PSD-95, Dlg, and ZO-1). PSD-95 possesses three PDZ domains. The second (PSD-95 PDZ2) interacts with NMDAR NR2 subunits at a seven-amino acid, COOH-terminal domain that contains a terminal tSXV motif (where S is serine, X is any amino acid, and V is valine) [15].

2. PDZ Domain-Mediated Protein Interactions in the CNS

PDZ domains were discovered from consensus sequences of 80 - 90 amino acid residues of three proteins: the postsynaptic density protein PSD-95, the *Drosophila* septate junction protein Dlg, and the tight junction protein ZO-1 [15]. The common structure of PDZ domains comprises six β strands (βA-βF) and two α helices (αA and αB). Peptide ligands from the extreme C-termini of targeted proteins bind as an antiparallel β-strand in a groove formed by the second α-helix (αB) and the second β-strand (βB) of the PDZ domains. Amino acid residues at the 0 and –2 positions of the carboxyl peptide play dominant roles in the peptide's binding to a cognate PDZ domain, although residues at the –1 and –3 positions and those further upstream also contribute to the binding. Despite similarities in secondary structure and the common preference for C-terminal ligands, PDZ domains display different binding specificity.

Generally, PDZ domains are classified into three types according to their specificity for C-terminal peptide ligands. In type I PDZ domains, such as those of PSD-95 and PSD-93, a serine or threonine residue occupies the –2 position of the C-terminal ligand. This type of PDZ domain is specific for the S/T-X-Φ target sequence (X: unspecified amino acid; Φ: hydrophobic amino acid) [17]. For instance, the type I PDZ domains mediate the protein interactions between the C-terminal ligand of NMDA receptor subunit NR2A/2B and the second PDZ domain of PSD-95 or PSD-93. In contrast, the type II PDZ domain (such as that of PICK1), specific for -X-Φ-X-Φ sequence, is characterized by hydrophobic residues at both the –2 position of the peptide ligand and the αB1 position of the PDZ domain [17]. For instance, the type II domain mediates the protein interactions between the C-terminal ligand of AMPA receptor subunit GluR2 and the PDZ domain of PICK1. The type III PDZ domain, such as that of neuronal nitric oxide synthase, is specific for a -X-D/E-X-Φ pattern and prefers negatively charged amino acids at the –2 position [18].

3. Mechanisms Underlying Tat-Mediated Intracellular Delivery

Cell surface heparan sulphate proteoglycans (HSPGs) have been shown to play a role in Tat-mediated intracellular delivery [19,20]. Tat-linked cargoes bind to HSPGs on the plasma membrane and are then taken up by endocytosis [21,22]. In the endocytosed vesicles, heparan sulphate is degraded by heparinase, which releases the Tat-linked cargoes [23]. The involvement of heparan sulphate in Tat-mediated intracellular delivery has been evidenced in three ways [21]. 1) Enzymatic removal of extracellular heparan sulphate drastically reduces cell uptake of Tat-linked cargoes; 2) The co-administration of exogenous heparan sulphate competitively inhibits the following cellular effects: the Tat uptake itself, the formation of aggregates on the cell membrane, and the reduction of the extracellular acidification rate; 3) The differential interference contrast image contrast of these aggregates on the membrane could be mimicked with a source of binding of exogenous heparan sulphate to the Tat.

Previous studies have suggested that Tat-linked cargoes enter cells via an energy-dependent endocytic process [24,25], because the membrane inhibitor sodium azide inhibits ATP production and impairs endocytosis [26]. The mechanism of entry by clathrin-coated vesicles has been ruled out. The receptor-independent endocytosis known as macropinocytosis has been demonstrated [27, 28]. It has been observed that Tat-linked cargoes are localized and sequestered in endosomes. Upon treatment with endosomal releasing polymer, poly(propylacrylic acid), the fusion cargoes are released into the cytoplasm [29]. However, the particular intracellular delivery pathway is dependent on characteristics of the cargo fused, conformation attained after fusion with Tat, and experimental conditions.

4. Tat-Mediated PDZ Peptide Delivery in Chronic Pain Treatment

The ability of Tat-linked cargoes to cross the blood-brain barrier has encouraged us to use this system in developing potential targets for chronic pain treatment (**Figure 1**). Our previous studies have demonstrated the roles of PDZ-containing scaffolding proteins (such as PSD-95) in the spinal transduction of NMDA receptor signaling in chronic pain states and found that deficiency of spinal PSD-95 significantly inhibits the development and maintenance of chronic pain [30,31]. To define further the role of PDZ domain-mediated NMDAR-PSD-95 protein interactions in chronic pain, we constructed a peptide comprising the PSD-95 PDZ2 and rendered it cell permeable by fusing it to Tat PTD to obtain the fusion peptide Tat-PSD-95 PDZ2. We injected mice intraperitoneally (systemically) or intrathecally (locally) with this fusion peptide and then assessed their behavioral responses to intraplantar injection of complete Freund's adjuvant (CFA) [32]. Importantly, we showed that Tat-PSD-95 PDZ2 was delivered into the spinal cord after

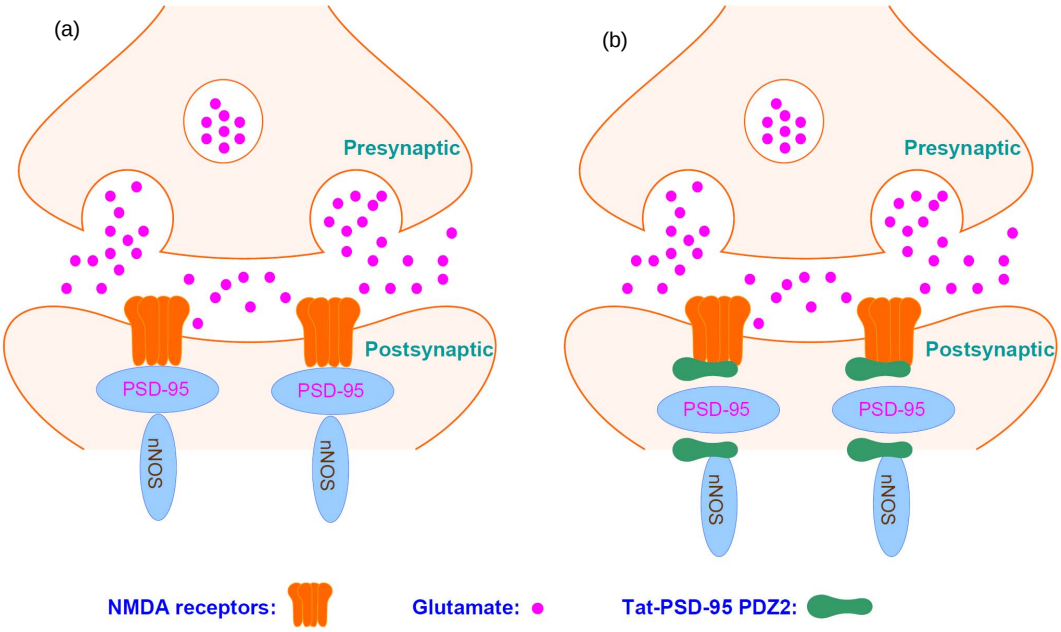

Figure 1. Tat-linked PDZ peptide disrupts NMDA receptor signaling in the central nervous system. (a) Under physiological condition, the scaffolding protein PSD-95 attaches NMDA receptors to internal signaling molecules at neuronal synapses by PDZ domain mediated protein-protein interactions with NMDA receptors and neuronal nitric oxide synthase (nNOS). Thus, PSD-95 might be involved in physiological and pathophysiological actions triggered via the activation of NMDA receptors; (b) The second PDZ domain of PSD-95 (PSD-95 PDZ2) interacts with the seven-amino acid, COOH-terminal domain containing a terminal tSXV motif (where S is serine, X is any amino acid, and V is valine) common to NR2 subunits of NMDA receptors. The PSD-95 PDZ2 also forms a heterodimeric PDZ-PDZ interaction with the PDZ domain of nNOS. Thus, Tat-linked PDZ peptide Tat-PSD-95 PDZ2 can disrupt these interactions and NMDA receptor signaling in the central nervous system. Because the activation of NMDA receptors plays an important role in the processing of nociceptive information, the disruption of PDZ domain-mediated protein-protein interactions within NMDA receptor signaling may inhibit the development of chronic pain.

intraperitoneal injection. Furthermore, the fusion peptide dose-dependently disrupted the protein-protein interactions between NMDAR NR2 subunits and PSD-95 and significantly inhibited CFA-induced chronic inflammatory pain [32]. These results suggest that PDZ domain-mediated protein interactions at spinal synapses might play an important role in the molecular mechanisms of chronic inflammatory pain behaviors. Our study provides novel insight into the molecular mechanisms that underlie chronic inflammatory pain states and a new approach for chronic inflammatory pain therapy. Thus, cell-permeable Tat peptides can treat chronic pain by disrupting PDZ domain-mediated protein-protein interactions. The Tat-linked PDZ peptides might be ready for clinical trials to develop a specific drug for chronic pain therapy.

5. Perspectives

In the last decade, the applications of the Tat-mediated intracellular delivery system have been expanded due to its several advantages, such as size-independent and non-viral transportation. This system can be improved if we modify the Tat PTD in order to make its delivery more specific, thereby widening its therapeutic potential. The ability to specifically deliver Tat-linked cargoes could theoretically reduce the side effects produced by the delivery of cargo to undesired organs and reduce the total amount of Tat-fused peptide drugs for CNS diseases including chronic pain.

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