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A CYCLIC PEPTIDE TARGETED AGAINST PSD-95 BLOCKS CENTRAL SENSITIZATION AND ATTENUATES THERMAL HYPERALGESIA

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Abstract—Post-synaptic density protein PSD-95 is emerging as a valid target for modulating nociception in animal studies. Based on the key role of PSD-95 in neuronal plasticity and the maintenance of pain behavior, we predicted that CN2097, a peptide-based macrocycle of nine residues that binds to the PSD-95 Discs large, Zona occludens 1 (PDZ) domains of PSD-95, would interfere with physiologic phenomena in the spinal cord related to central sensitization. Furthermore, we tested whether spinal intrathecal injection of CN2097 attenuates thermal hyperalgesia in a rat model of sciatic neuropathy. Results demonstrate that spinal CN2097 reverses hyperexcitability of wide dynamic range (WDR) neurons in the dorsal horn of neuropathic rats and decreases their evoked responses to peripheral stimuli (brush, low caliber von Frey and pressure), whereas CN5125 ("negative control") has no effect. CN2097 also blocks C-fiber long-term potentiation (LTP) in the dorsal horn, which is linked to neuronal plasticity and central sensitization. At a molecular level, CN2097 attenuates the increase in phosphorylated p38 MAPK, a key intracellular signaling pathway in neuropathic pain. Moreover, spinal injection of CN2097 blocks thermal hyperalgesia in neuropathic rats. We conclude that CN2097 is a small molecule peptide with putative anti-nociceptive effects that modulates physiologic phenomena related to central sensitization under conditions of chronic pain.

Key words: pain, PSD-95, hyperalgesia, WDR, LTP, p38.

Protein-protein interactions that are critical for normal and pathological signal transduction pathways have recently become attractive targets for developing therapeutic molecules. In particular, post-synaptic density protein PSD-95 has been identified mainly in dorsal horn laminae I and II, with distinct expression and localization patterns suggesting predominant expression in nociceptive pathways and a key role in pain mechanisms (Garry et al., 2003; Tao et al., 2000), though a more recent study indicated widespread expression of PSD-95 at glutamatergic synapses throughout the spinal gray matter (Polgar et al., 2008). Persistent or recurrent pain caused by nerve damage is referred to as neuropathic, which tends to be mostly intractable (Campbell and Meyer, 2006; Dworkin et al., 2003) and associated with neuropathic behavior, including thermal hyperalgesia (increased pain evoked by mildly noxious heat) (Treede et al., 1992). Based on studies using genetic approaches, PSD-95 emerged as an important factor in the maintenance of thermal hyperalgesia, (Tao et al., 2000, 2001, 2003). For example, a synthetic fusion protein of 168 residues, comprising the entire PDZ2 (PSD-95, Discs large, Zona occludens 1) domain of PSD-95, was found to significantly reduce chronic pain behavior in mice following injection of complete Freund's adjuvant in the hindpaw (Tao et al., 2008). More recently, it was reported that thermal hyperalgesia is attenuated by disruption of the protein–protein interaction between nNOS and PSD-95, using a small molecule inhibitor and a cell permeable Tat fusion protein (Florio et al., 2009). Therefore, PSD-95 is thought to be a novel molecular target for managing chronic pain and several other neuropathologic conditions (Gardoni, 2008; Houslay, 2009; Tao and Johns, 2006; Tao and Raja, 2004).

Therefore, with the aim of targeting the PDZ domains of PSD-95, we designed and synthesized compound CN2097 (Fig. 1), a ligand directly based on a macrocyclic peptide previously established as capable of binding PDZ3 (Li et al., 2004) and PDZ1 (Piserchio et al., 2004) domains of PSD-95. Cell-based evaluation of this precursor macrocyclic peptide demonstrated activity within a PDZ domain-based clustering assay, and also exhibited enhanced longevity of action over a linear peptide control, suggesting added stability imparted by the cyclic structure (Piserchio et al., 2004). CN2097 was generated by appending a sequence of seven arginines via a Cys–Cys disulfide linkage. This modification was adopted to impart cell permeability, since the use of polyarginine tags has been shown to be effective at delivering attached compounds across cellular membranes (Rothbard et al., 2000). In addition, we designed CN5125, an analog of CN2097 in which the key binding determinants at positions "0" and "−2" (Val and Thr, respectively, in Fig. 1) are both replaced with Gly residues. Glycine at those positions dramatically reduces binding to PDZ domains as determined in previously reported biochemical binding studies (Saro et al., 2007).

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Abbreviations: CCI, chronic constriction injury; PDZ, PSD-95 Discs large, Zona occludens 1; PSD, post-synaptic density protein; PWL, paw withdrawal latencies; TMR, tetramethylrhodamine; TS, tetanic stimulation; WDR, wide dynamic range.

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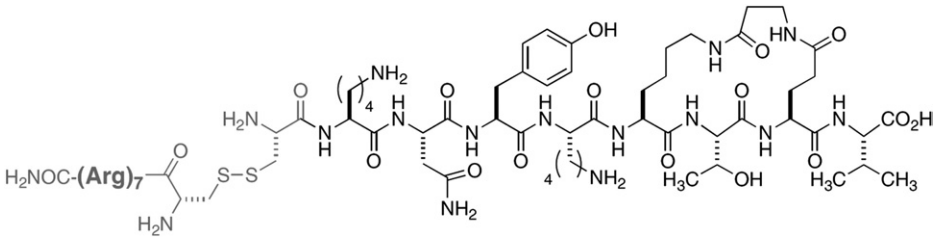

Fig. 1. Structure of cellular probe CN2097.

CN5125 was thus used as the corresponding "negative control."

Experiments with PDZ domain arrays indicated that the biotinylated analog of the CN2097 precursor selectively binds with only the PDZ1-2 and PDZ3 domains of PSD-95, interacting minimally, or not at all, with the other 94 PDZ domain constructs in the array (Fig. 2). Compared to the previously reported Tat fusion proteins that bind PSD-95 and that exert anti-nociceptive properties (Florio et al., 2009; Tao et al., 2008), CN2097 and its related analogues are relatively much smaller peptides in size, which were designed and synthesized based on a biochemical rationale rather than high throughput protein screening for binding against PSD-95 and/or its partner molecules. In addition, we here investigate some of the putative anti-nociceptive mechanisms of CN2097 at behavioral, cellular and molecular levels.

Because PSD-95 plays a key role in neuronal plasticity, which is a core component of learning and memory (Hata and Takai, 1999; Steiner et al., 2008), as well as central sensitization under chronic pain conditions (Garry et al., 2003; Ji and Woolf, 2001; Treede et al., 1992; Woolf, 2007), we predicted that CN2097 would modulate physiological processes related to central sensitization and long-term plasticity in an animal model of chronic neuropathic pain. Furthermore, we tested the anti-nociceptive effects of CN2097 on thermal hyperalgesia in neuropathic rats. Confirming our predictions, our data suggest that CN2097

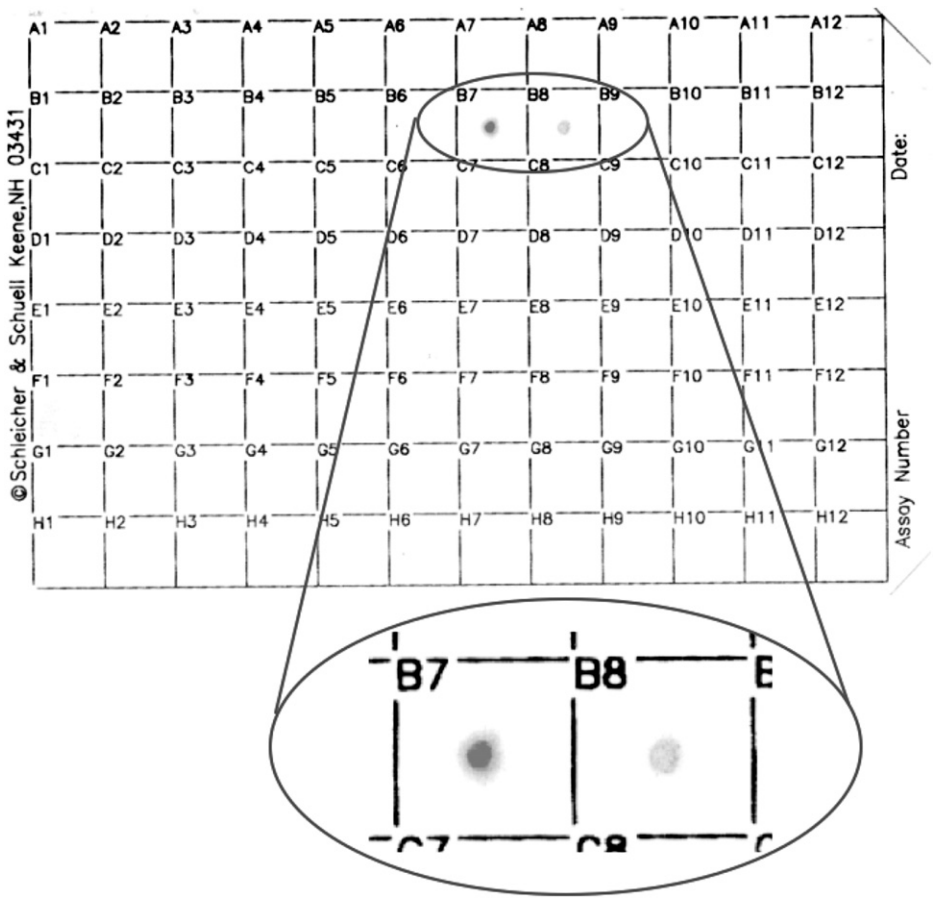

Fig. 2. PDZ domain array. PDZ domain array probed with the CN5115, a TMR-labeled analog containing the PDZ domain-targeting portion of CN2097 (Arrays provided by Dr. Randy Hall, Emory University).