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Perspective

Increasing spinal 5-HT$_{2A}$ receptor responsiveness mediates anti-allodynic effect and potentiates fluoxetine efficacy in neuropathic rats. Evidence for GABA release

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ABSTRACT

Antidepressants are one of the first line treatments for neuropathic pain but their use is limited by the incidence and severity of side effects of tricyclics and the weak effectiveness of selective serotonin reuptake inhibitors (SSRIs). Serotonin type 2A (5-HT$_{2A}$) receptors interact with PDZ proteins that regulate their functionality and SSRI efficacy to alleviate pain. We investigated whether an interfering peptide (TAT-2ASCV) disrupting the interaction between 5-HT$_{2A}$ receptors and associated PDZ proteins would improve the treatment of traumatic neuropathic allodynia. Tactile allodynia was assessed in spinal nerve ligation-induced neuropathic pain in rats using von Frey filaments after acute treatment with TAT-2ASCV and/or 5-HT$_{2A}$ receptor agonist, alone or in combination with repeated treatment with fluoxetine. *In vivo* microdialysis was performed in order to examine the involvement of GABA in TAT-2ASCV/fluoxetine treatment-associated analgesia. TAT-2ASCV (100 ng, single i.t. injection) improved SNL-induced tactile allodynia by increasing 5-HT$_{2A}$ receptor responsiveness to endogenous 5-HT. Fluoxetine alone (10 mg/kg, five i.p. injections) slightly increased tactile thresholds and its co-administration with TAT-2ASCV (100 ng, single i.t. injection) further enhanced the anti-allodynic effect. This effect depends on the integrity of descending serotonergic bulbospinal pathways and spinal release of GABA. The anti-allodynic effect of fluoxetine can be enhanced by disrupting 5-HT$_{2A}$ receptor-PDZ protein interactions. This enhancement depends on 5-HT$_{2A}$ receptor activation, spinal GABA release and GABAA receptor activation.

Abbreviations: ANOVA, analysis of variance; α-methyl-5-HT, alpha-methyl-5-hydroxy-tryptamine maleate; AUC, area under the curve; CCI, chronic constriction injury; DMSO, dimethylsulfoxyde; GABA, gamma amino butyric acid; HPLC, high performance liquid chromatography; i.m., intramuscular; i.p., intraperitoneal; i.t., intrathecal; LDH, lumbar disc herniation; MRM, Multiple Reaction Monitoring; M100907, ((R)-(+)-a-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-pipidinemethanol); PAG, peri-aqueductal gray; PDZ, post-synaptic density protein (PSD-95), *Drosophila* disc large tumor suppressor (Dlg1) Zonula occludens-1 protein (zo-1); RVM, rostro ventromedial medulla; s.c., sub-cutaneous; SEM, standard error of the mean; SNL, spinal nerve ligation; SNRI, serotonin and norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; TAT-2ASCV, YGRKKRRQRRRTVNEKVSCV; TAT-2ASCA, YGRKKRRQRRRTVNEKVSCA; TCA, tricyclic antidepressant; 5-HT, 5-hydroxy-tryptamine; 5,7-DHT, 5,7-dihydroxytryptamine creatinin sulfate.

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1. Introduction

Neuropathic pain is initiated or caused by a lesion or disease of the somatosensory system [1,2]. Unconventional analgesics such as tricyclic antidepressants (TCA) have been recommended as first-line treatments for many years, but only a minority of patients experiences a clinically relevant benefit from such treatments [3,4]. Selective serotonin (5-HT) reuptake inhibitor (SSRI) antidepressants, that are safer than TCA, are poorly effective [5,6]. Consistent with clinical findings, some pre-clinical studies have shown that the antinociceptive/antihyperalgesic effect of serotonin (5-HT receptor ligands) is less potent in neuropathic than in acute pain models [7–9]. The 5-HT_{2A} receptor has been identified as one of the 5-HT receptors contributing to 5-HT-induced analgesia in various pain conditions. For example, central 5-HT_{2A} receptor activation inhibits responses of wide dynamic range neurons [10] and reduces craniofacial [11] and peripheral [12] hyperalgesia induced by nerve ligation [12–15]. In certain conditions, antinociception induced by SSRIs such as fluvoxamine [16,17] and fluoxetine [18] as well as pain relief induced by the serotonin and norepinephrine reuptake inhibitor (SNRI) milnacipran [19] are mediated by 5-HT_{2A} receptor activation. The intracellular C-terminus of these receptors interacts with intracellular proteins including a majority of PDZ [Post synaptic density protein (PSD-95), Drosophila disc large tumor suppressor (Dlg1), and zonula occludens-1 protein (zo-1)] proteins [20]. These proteins participate in receptor trafficking, localization, internalization, desensitization regulation and in the fine tuning of G protein activation [21].

We previously showed that disrupting 5-HT_{2A} receptor-PDZ protein interactions induced an antihyperalgesic effect in diabetes-induced neuropathic pain [9] and carrageenan-induced inflammatory pain [22] in rats. This disruption was selectively achieved by intrathecally injecting an interfering peptide comprising the nine C-terminal residues of the 5-HT_{2A} receptor fused with the transduction domain of HIV type 1 Tat protein (amino acid sequence YGRKKRRQRRRTVNEKVSC, TAT-2ASCV) to allow its transduction into spinal neurons *in vivo*. In both conditions, the analgesic effect of the peptide was selectively abolished by the administration of a 5-HT_{2A} receptor antagonist (M100907) but not with the highly selective 5-HT_{2C} receptor antagonist SB242084 [23]. Moreover, when combined with fluoxetine in diabetic neuropathic rats, the peptide produced a marked and sustained antihyperalgesic effect [9]. The peptide also revealed 5-HT_{2A} receptor-operated calcium responses in cultured neurons [9]. Further studies showed that small molecules that interact with the PDZ1 or PDZ2 domains of the PSD-95 protein induce a dose-dependent antihyperalgesic effect in neuropathic rats [24,25].

We wondered whether disruption of spinal 5-HT_{2A} receptor PDZ protein interactions might likewise alleviate tactile allodynia induced by peripheral nerve ligation. Accordingly, we explored whether the peptidyl mimetic strategy that disrupts the interactions between the receptor's PDZ-binding motif and the associated PDZ proteins would be efficacious in relieving traumatic neuropathic pain. To address this issue, we examined the effect of spinal injection of the TAT-2ASCV peptide on tactile allodynia induced by spinal nerve ligation (SNL) in rats. We also investigated the impact of TAT-2ASCV peptide administration on the anti-allodynic response of fluoxetine in SNL rats. Finally, we analyzed the underlying mechanism of this effect by examining the involvement of 5-HT_{2A} receptors and serotonergic bulbospinal pathways, and by measuring spinal extracellular levels of GABA using *in vivo* microdialysis in awake rats.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (175–200 g body weight, approximately 35 days old the day of their arrival) were used (Janvier, Le Genest St Isle, France). Animals were housed in specific pathogen free area in standard cages (four per cage) and were maintained on a 12:12 h light/dark cycle under standard conditions. In addition, food and water were available *ad libitum*.

All experiments involving animals were performed in accordance with the ARRIVE guidelines [26,27]. All procedures and animal handling were carried out in accordance with the European Commission's directive 2010/63/EU and were approved by the local Ethics Committee of Auvergne (C2EA, France). Great care was taken to avoid or minimize discomfort of the animals.

2.2. Spinal nerve ligation surgery

Neuropathy was induced as previously described [28]. Briefly, under xylazine/ketamine anesthesia (Rompun 2%, 10 mg/kg i.p., Imalgène 1000, 75 mg/kg i.p.), left spinal nerve L5 was exposed and tightly ligated with 4-0 silk suture (Mersilk, Ethicon LLC Johnson & Johnson, San Lorenzo, Puerto Rico). The muscle and skin were then sutured with 5-0 monofilament (Monocryl, Ethicon LLC Johnson & Johnson, San Lorenzo, Puerto Rico) and Michel's suture clips (A75 Perfect, Ets Bruneau, Bretigny-sur-Orge, France), respectively. After surgery, animals were treated with antibiotic spray (Thiamphenicol, 0.095 mg/ml) and non-steroidal anti-inflammatory drug (Meloxicam, 2 mg/kg s.c., one injection per day for 2 days) and were isolated until waking. Sham animals underwent the same surgery except for the ligation itself. The animals were allowed to recover and were monitored routinely to insure good health.

Animals showed good health, regular body weight gain and normal behavior. Clinical postoperative examination showed that wound margins were healed by day 8 after the surgery. Signs of disability and distress were absent: no evidence of back pain (48 h after surgery) was observed neither prostrated behavior after surgery. Animals did not develop post-operative sensory loss or motor deficit as a consequence of surgery.

2.3. Behavioral assessment: tactile allodynia

Rats were placed individually in plexiglas compartments ($17 \times 11 \times 13$ cm) with a wire mesh bottom that allowed full access to paws. After 20 min of habituation, paw-withdrawal threshold was measured and 50% response threshold was calculated using the Up-Down method and Dixon's formulae as previously described [29]. A series of 8 von Frey monofilaments with logarithmic incremental stiffness (of 1.4, 2, 4, 6, 8, 10, 15 and 26 g) was used. First hair applied was the 6 g hair and was pressed perpendicularly against the plantar surface of the ipsilateral (side of surgery) hind paw with sufficient force to bend the filament, for 6 s. Paw withdrawal or licking was considered as a positive response and the next weaker filament was applied. In case of negative response (no paw withdrawal or licking), we then applied the next stronger filament. This paradigm continued until four measurements have been obtained after an initial change of behavior, or until four consecutive negative responses or five consecutive positive responses.

2.4. Intrathecal injection

Intrathecal (i.t.) injections were performed under isoflurane anaesthesia (4% induction, 2% maintenance) according to the method previously described [30]. The anesthetized rat was held