Disrupting 5-HT$_{2A}$ Receptor/PDZ Protein Interactions Reduces Hyperalgesia and Enhances SSRI Efficacy in Neuropathic Pain

Xavier Pichon[1,2], Anne S Wattiez[1,2], Carine Becamel[3–5], Ingrid Ehrlich[6], Joel Bockaert[3–5], Alain Eschalier[1,2,7], Philippe Marin[3–5] and Christine Courteix[1,2]

[1]Clermont Université, Université d'Auvergne, Pharmacologie fondamentale et clinique de la Douleur, Clermont-Ferrand, France; [2]Inserm, U766, Clermont-Ferrand, France; [3]Institut de Génomique Fonctionnelle, Universités de Montpellier, CNRS UMR 5203, Montpellier, France; [4]INSERM U661, Montpellier, France; [5]CNRS UPR 9023, Montpellier Cedex 5, France; [6]Hertie Institute for Clinical Brain Research, Tübingen, Germany; [7]CHU Clermont-Ferrand, Service de pharmacologie, Clermont-Ferrand, France

Antidepressants are one of the first-line treatments for neuropathic pain. Despite the influence of serotonin (5-hydroxytryptamine, 5-HT) in pain modulation, selective serotonin reuptake inhibitors (SSRIs) are less effective than tricyclic antidepressants. Here, we show, in diabetic neuropathic rats, an alteration of the antihyperalgesic effect induced by stimulation of 5-HT$_{2A}$ receptors, which are known to mediate SSRI-induced analgesia. 5-HT$_{2A}$ receptor density was not changed in the spinal cord of diabetic rats, whereas postsynaptic density protein-95 (PSD-95), one of the PSD-95/disc large suppressor/zonula occludens-1 (PDZ) domain containing proteins interacting with these receptors, was upregulated. Intrathecal injection of a cell-penetrating peptidyl mimetic of the 5-HT$_{2A}$ receptor C-terminus, which disrupts 5-HT$_{2A}$ receptor–PDZ protein interactions, induced an antihyperalgesic effect in diabetic rats, which results from activation of 5-HT$_{2A}$ receptors by endogenous 5-HT. The peptide also enhanced antihyperalgesia induced by the SSRI fluoxetine. Its effects likely resulted from an increase in receptor responsiveness, because it revealed functional 5-HT$_{2A}$ receptor-operated Ca^{2+} responses in neurons, an effect mimicked by knockdown of PSD-95. Hence, 5-HT$_{2A}$ receptor/PDZ protein interactions might contribute to the resistance to SSRI-induced analgesia in painful diabetic neuropathy. Disruption of these interactions might be a valuable strategy to design novel treatments for neuropathic pain and to increase the effectiveness of SSRIs.

INTRODUCTION

Serotonin (5-hydroxytryptamine, 5-HT) is a key modulator of spinal nociceptive transmission.[1] Its predominant inhibitory role on persistent pain has definitely been established using mice lacking central 5-HT neurons (Lmx1b$^{f/f/p}$ mice). These mice exhibit enhanced persistent inflammatory pain, which is attenuated by intrathecal injection of 5-HT.[2] Moreover, the analgesic effects of the selective serotonin reuptake inhibitor (SSRI) fluoxetine are abolished, indicating that pain relief by SSRI antidepressants is completely dependent on the central 5-HT system. However, 5-HT is much less potent against persistent neuropathic pain than against acute nociception,[3] consistent with clinical studies indicating that SSRIs are less efficient than mixed 5-HT-norepinephrine reuptake inhibitors for treating chronic pain.[4]

The analgesic effects of SSRIs are strongly dependent of spinal 5-HT$_{2A}$ receptor activation.[5,6] Moreover, selective activation of spinal 5-HT$_{2A}$ receptors suppresses allodynia in a rat model of spinal nerve ligation[7] and the reaction to inflammatory stimuli.[8] 5-HT$_{2A}$ receptors expressed on spinal GABAergic neurons[9] would mediate antinociceptive actions, consistent with the induction of GABAergic/glycinergic inhibitory potentials in the spinal cord upon 5-HT$_{2A}$ receptor stimulation.[10] We hypothesize that the weak effectiveness of SSRIs in persistent pain might result from an alteration of 5-HT$_{2A}$ receptor-mediated analgesic effects.

5-HT$_{2A}$ receptors interact with a variety of intracellular proteins, which are essential for the regulation of their functional status.[11,12] These include postsynaptic density protein-95 (PSD-95)/disc large suppressor/zonula occludens-1 (PDZ) domain containing proteins (PDZ proteins) of the membrane-associated guanylate kinase (MAGUK) family.[13] Several studies have revealed a role of MAGUKs in chronic neuropathic pain. Knockdown of spinal PSD-95 attenuates N-methyl-D-aspartate (NMDA)-induced thermal hyperalgesia and nerve injury-induced mechanical and thermal hyperalgesia during the development and maintenance of chronic neuropathic pain in rats.[14–16] Mutant mice expressing truncated PSD-95 exhibit a complete lack of reflex sensitization to mechanical and thermal nociceptive stimuli following chronic constriction of the sciatic nerve.[17] Spinal PSD-93 knockdown in rats and knockout in mice prevent NMDA receptor-dependent persistent pain induced by spinal nerve injury.[18,19] We reasoned

that association of MAGUKs with spinal 5-HT$_{2A}$ receptors might also influence regulation of nociceptive transmission and contribute to both neuropathic pain-related behaviors and the weak effectiveness of SSRIs in this context.

Here, we have examined the effect of spinal 5-HT$_{2A}$ receptor stimulation and the influence of its interaction with PDZ proteins toward mechanical and thermal noxious stimuli in hyperalgesic diabetic neuropathic rats. We demonstrate a lack of sensitivity of diabetic hyperalgesic rats to antinociceptive effects induced by 5-HT$_{2A}$ receptor stimulation. This phenomenon reflected association of 5-HT$_{2A}$ receptors with PDZ proteins rather than receptor downregulation. Indeed, disrupting 5-HT$_{2A}$ receptor/PDZ protein interactions induced an antihyperalgesic effect in diabetic rats and strongly enhanced antihyperalgesia produced by fluoxetine.

RESULTS

Decreased sensitivity of diabetic hyperalgesic rats to antinociceptive effects induced by 5-HT$_{2A}$ receptor stimulation

We first explored the influence of 5-HT$_{2A}$ receptor stimulation against mechanical and thermal nociception in healthy rats. Intrathecal injection of α-methyl-5-hydroxytryptamine (α-methyl-5-HT) (100 μg/rat), a 5-HT$_{2A}$ receptor agonist, increased paw-pressure-induced vocalization thresholds (VT) 15 and 30 minutes after injection (**Figure 1a,b**). This antinociceptive effect did involve 5-HT$_{2A}$ receptors, as it was abolished by the coinjection of the "selective" 5-HT$_{2A}$ receptor antagonist M100907 [150 ng/rat intrathecally (i.t.), **Figure 1c**], which by itself did not modify VT. It was also suppressed by the coinjection of bicuculline (3 μg/rat i.t., **Figure 1d**), a γ-aminobutyric acid (GABA)-A receptor antagonist, indicative of implication of GABAergic interneurons in the antinociceptive effect induced by 5-HT$_{2A}$ receptor activation. Intrathecal injection of α-methyl-5-HT (100 μg/rat) suppressed the spontaneous place preference behavior for the 25 °C versus the 45 °C surface (**Figure 1e**) in the two-temperature choice test,[20] indicating that 5-HT$_{2A}$ receptor activation also induces analgesia against thermal noxious stimuli.

In contrast, α-methyl-5-HT (100 μg/rat i.t.) did not exert any antihyperalgesic action against mechanical and thermal noxious stimulations in diabetic hyperalgesic rats, as shown by the lack of significant increase of paw pressure-induced VT (**Figure 1a,b**) or the lack of abolition of the temperature preference behavior (**Figure 1e**). [^{3}H]-ketanserin saturation analysis performed on lumbar spinal cord membranes from healthy and diabetic hyperalgesic rats showed that neither affinity nor receptor density were altered in diabetic hyperalgesic rats (Kd = 1.81 ± 0.17 nmol/l, B_{max} = 57.6 ± 4.1 fmol/mg protein, $n = 4$ versus Kd = 2.03 ± 0.09 nmol/l, B_{max} = 59.2 ± 4.8 fmol/mg protein, $n = 4$, in healthy rats). These results indicated that the alteration of 5-HT$_{2A}$ receptor-mediated antinociception in diabetic hyperalgesic rats did not result from receptor downregulation but rather from a decrease in receptor responsiveness.

Identification of PDZ proteins interacting with 5-HT$_{2A}$ receptors in spinal cord of healthy and diabetic rats

We hypothesized that interaction of spinal 5-HT$_{2A}$ receptors with MAGUKs, which have been involved in inflammatory[21] and neuropathic pain,[14,15,17–19] could contribute to the alteration of 5-HT$_{2A}$
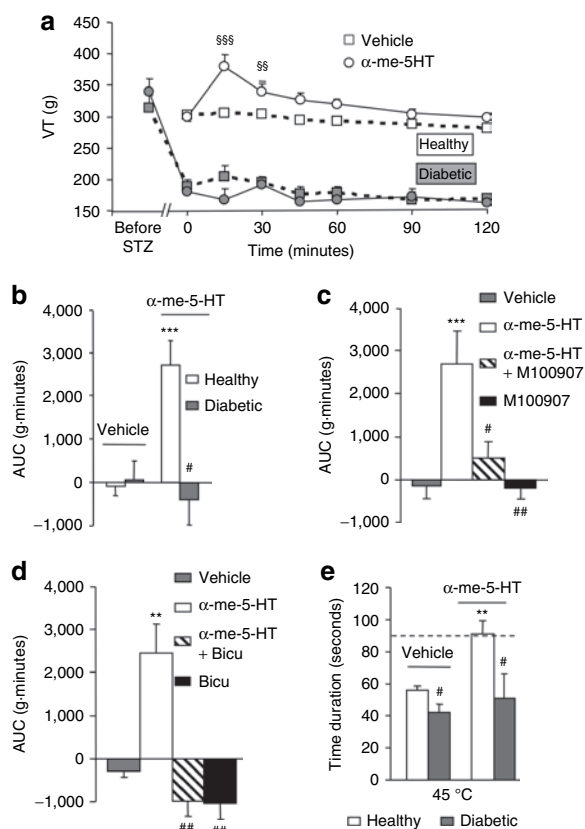

Figure 1 Resistance of diabetic hyperalgesic rats to 5-HT$_{2A}$ receptor-mediated antinociceptive effect. (**a**) Effect of α-methyl-5-HT (α-me-5-HT, 100 μg/rat i.t.) or NaCl (vehicle, 10 μl/rat i.t.) in healthy and diabetic hyperalgesic rats. Mechanical sensitivity was determined by measuring paw-pressure induced vocalization thresholds (VT). $^{§§}P < 0.01$ and $^{§§§}P < 0.001$ compared with values measured before α-me-5-HT/vehicle injection (time 0). (**b**) Area under the time-course (0–60 minutes) curve (AUC) of the vocalization threshold variations, calculated by the trapezoidal rule and expressed as means ± SEM of values obtained from 20 to 21 healthy and 6 to 10 diabetic rats. ***$P < 0.001$ versus vehicle-treated group. #$P < 0.05$ versus corresponding healthy group. (**c**) Inhibition of the antinociceptive effect of α-me-5-HT (100 μg/rat i.t.) by M100907 (150 ng/rat i.t.) in healthy rats ($n = 8$–12), assessed by the paw-pressure test. ***$P < 0.001$ versus vehicle. #$P < 0.05$, ##$P < 0.01$ versus α-me-5-HT. (**d**) Inhibition of the antinociceptive effect of α-me-5-HT (100 μg/rat i.t.) by bicuculline (Bicu, 3 μg/rat i.t.) in healthy rats ($n = 6$–8). **$P < 0.01$ versus vehicle. ##$P < 0.01$ versus α-me-5-HT assessed by the paw-pressure test. (**e**) Effect of α-me-5-HT (100 μg/ rat i.t.) or vehicle (10 μl/rat i.t.) on the two-temperature choice test in healthy and diabetic rats. Thirty minutes after injection, α-me-5-HT induces a behavioral bias for occupying the 45 °C surface in healthy but not diabetic rats, during a 3-minute trial (180 seconds). **$P < 0.01$ versus vehicle. #$P < 0.05$ versus the corresponding healthy group. The dashed line represents the theoretical mean in absence of preference behavior (90 seconds spent on each surface). i.t., intrathecal; α-me-5-HT, α-methyl-5-hydroxytryptamine.

receptor functionality in diabetic neuropathy. To determine whether 5-HT$_{2A}$ receptors interact with MAGUKs in the spinal cord and to identify in a global manner these proteins, we used a proteomic approach, which has already proved efficiency and sensitivity for characterizing specific PDZ-binding partners of 5-HT receptors in the brain.[13] We identified five protein partners of the 5-HT$_{2A}$ receptor in spinal cord extracts from healthy and diabetic hyperalgesic rats (**Supplementary Figure S1** and **Table S1**). These include SAP97, PSD-95, and MAGUK p55 subfamily member 2 (MPP2), three

MAGUKs, and MAGI2, a MAGUK with inverted domains. These proteins, except for MPP2, were previously identified as PDZ partners of brain 5-HT$_{2A}$ receptors.[13] The 5-HT$_{2A}$ receptor C-terminus also recruited Sec23A, one subunit of the coat complex II (COPII), involved in protein export from endoplasmic reticulum to either the endoplasmic reticulum–Golgi intermediate compartment or to the Golgi complex. Although Sec23A lacks any PDZ domains, the PDZ-binding motif located at the 5-HT$_{2A}$ receptor C-terminus contributed to its interaction with the receptor (**Supplementary Figure S1a**).

Proteomic analysis also revealed that the amount of PSD-95 (the first MAGUK that has been implicated in chronic pain[14–16,18,19]) recruited by the 5-HT$_{2A}$ receptor C-terminus in spinal cord was greater in diabetic hyperalgesic rats than in healthy rats (**Supplementary Figure S1a**). Correspondingly, western blotting experiments indicated an increase in PSD-95 expression in dorsal spinal cord of diabetic hyperalgesic rats, compared with healthy rats ($+52 \pm 4\%$, $n = 4$, **Supplementary Figure S1b**).

Disruption of 5-HT$_{2A}$ receptor/PDZ protein interactions suppresses hyperalgesia in diabetic rats

We next evaluated the overall contribution of 5-HT$_{2A}$ receptor/PDZ protein interactions to pain sensitivity in diabetic neuropathic rats, using a peptide comprising the nine C-terminal residues of the 5-HT$_{2A}$ receptor that competes for interactions between the receptor's PDZ-binding motif and target PDZ proteins. This peptide was fused N-terminally with the transduction domain of human immunodeficiency virus type 1 Tat protein (TAT-2ASCV) to allow its intracellular delivery.[22] Pull-down experiments showed that TAT-2ASCV, but not the corresponding peptide in which the C-terminal valine was substituted by an alanine (TAT-2ASCA), efficiently prevented association between the receptor and its MAGUK partners PSD-95 and SAP97 *in vitro* (**Supplementary Figure S2a**). The ability of these peptides to transduce into spinal neurons following intrathecal injection was verified by using methanethiosulfonate (MTS)-4-fluorescein-labeled peptides. Fluorescent labeling of spinal cord cells was detectable for 1 hour after intrathecal injection of MTS-4-TAT-2ASCV, but not following injection of the corresponding peptide lacking the Tat transduction domain (MTS-4-TAT-empty-2ASCV, **Supplementary Figure S2b**). Moreover, intrathecal injection of TAT-2ASCV decreased coimmunoprecipitation of PSD-95 and SAP97 with 5-HT$_{2A}$ receptors in spinal cord extracts (**Supplementary Figure S2c**), indicating that TAT-2ASCV inhibited interaction between 5-HT$_{2A}$ receptor and its MAGUK partners *in vivo*. In contrast, injection of TAT-2ASCA did not inhibit interaction between spinal 5-HT$_{2A}$ receptor and PDZ proteins (**Supplementary Figure S2c**). This peptide was further used as control in behavioral studies.

Intrathecal injection of TAT-2ASCV had no effect on mechanical nociception in healthy rats (**Supplementary Figures S3b,c**) but dose-dependently (3–30 ng/rat) increased VT in diabetic hyperalgesic rats, as shown by measuring the time course of the paw pressure-induced VT and the area under the curves (AUC) of the threshold variations (**Figure 2a,b**). A maximal antihyperalgesic effect was observed 30 minutes after injection (**Figure 2a**). Intrathecal injection of the TAT-2ASCA control peptide did not induce any antihyperalgesic action (**Figure 2a,b**). Further
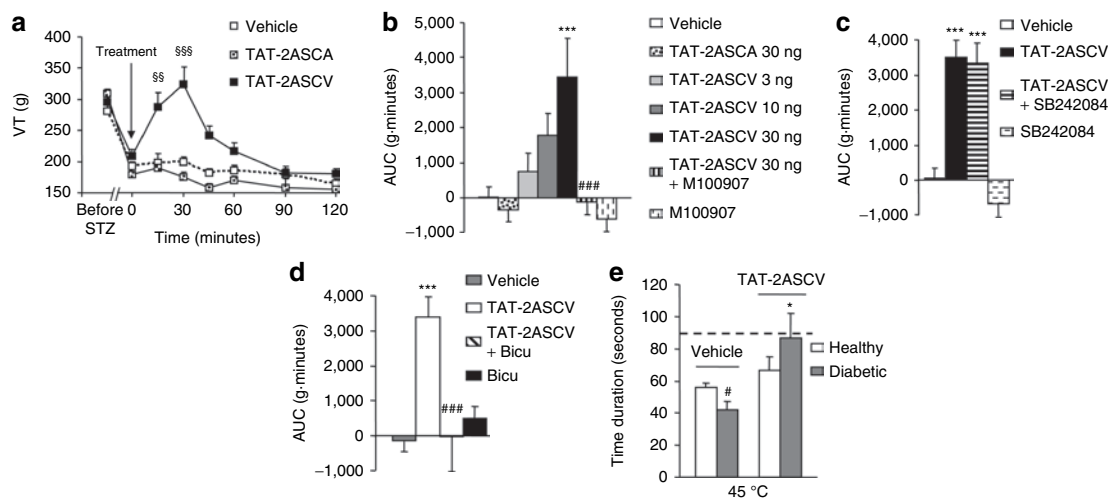

Figure 2 Disruption of 5-HT$_{2A}$ receptor/PDZ protein interactions produces antihyperalgesic effects in diabetic neuropathic rats. (**a**) Diabetic hyperalgesic rats were injected with either the TAT-2ASCV or the TAT-2ASCA peptide (30 ng/rat i.t., each) or vehicle (10 μl/rat i.t.). The vocalization thresholds to paw-pressure were measured up to 120 minutes after the injection. $^{§§}P < 0.01$ and $^{§§§}P < 0.001$, compared with values measured before the peptide/vehicle injection (time 0). (**b**) Diabetic rats were injected with the indicated doses of the TAT-2ASCV peptide or with the TAT-2ASCA peptide (30 ng/rat i.t.) or with M100907 (150 ng/rat i.t.) in the absence or presence of the TAT-2ASCV peptide (30 ng/rat, i.t.). Data, expressed as area under the time-course (0–60 minutes) curve (AUC) of vocalization threshold variations, are means ± SEM of values obtained in 6–12 rats. $^{***}P < 0.01$, versus vehicle. $^{###}P < 0.001$ versus TAT-2ASCV (30 ng/rat)-treated group. (**c**) Diabetic rats were injected with SB242084 (150 ng/rat i.t.) in the absence or presence of the TAT-2ASCV peptide (30 ng/rat, i.t.). Data, expressed as in **b**, are means ± SEM of values obtained in six rats. $^{***}P < 0.01$, versus vehicle. (**d**) Inhibition of the antihyperalgesic effect of the TAT-2ASCV peptide (30 ng/rat i.t.) by bicuculline (Bicu, 3 μg/rat i.t.) in diabetic rats ($n = 10–17$). $^{***}P < 0.001$ versus vehicle. $^{###}P < 0.001$ versus TAT-2ASCV-treated group; data are expressed as **b**. (**e**) Thirty minutes after injection, the TAT-2ASCV peptide (30 ng/rat i.t.) suppressed thermal hyperalgesia in diabetic rats, as shown by the increase in time duration spent on the plate set at 45 °C ($n = 4–7$ rats), see legend of **Figure 1e**. $^{*}P < 0.05$, compared with values measured before the peptide/vehicle injection (time 0). i.t., intrathecal; VT, vocalization thresholds.

supporting that the effect of TAT-2ASCV was due to the inhibition of 5-HT$_{2A}$ receptor/PDZ protein interactions, its effect on mechanical hyperalgesia was entirely inhibited by the coadministration of M100907 (150 ng/rat i.t., **Figure 2b**), whereas it was not affected by the 5-HT$_{2C}$ receptor antagonist SB242084 (150 ng/rat i.t., **Figure 2c**). Finally, inhibition of mechanical hyperalgesia by TAT-2ASCV was abolished by the coadministration of the GABA-A receptor antagonist bicuculline (3 µg/rat i.t., **Figure 2d**). The maximally effective dose of TAT-2ASCV against mechanical hyperalgesia (30 ng/rat) also suppressed the spontaneous temperature preference behavior in diabetic hyperalgesic rats, whereas it did not induce analgesic effect against noxious thermal stimulus in healthy animals (**Figure 2e**).

Intrathecal injection of TAT-2ASCV (30 ng/rat) did not prevent co-immunoprecipitation of PSD-95 with NMDA receptors in spinal cord extracts (**Supplementary Figure S3a**), suggesting that this peptide specifically inhibited interaction of MAGUKs with 5-HT$_{2A}$ receptors but not their association with other receptors known to bind to these PDZ proteins and to be involved in pain processing. Further supporting its specificity toward 5-HT$_{2A}$ receptor-mediated antihyperalgesia, injection of TAT-2ASCV did not affect NMDA-induced mechanical hypersensitivity in healthy rats (**Supplementary Figures S3b,c**). Furthermore, rats showed normal motor activity after administration of TAT-2ASCV, as assessed by their spontaneous motor activity, grip strength, or righting score (**Supplementary Figures S3d–f**), indicating that the peptide did not affect motor functions. Collectively, our observations indicate that disruption of 5-HT$_{2A}$ receptor/PDZ protein interactions reveals an antihyperalgesic action of 5-HT$_{2A}$ receptors activated by endogenous 5-HT in diabetic neuropathy.

Disruption of 5-HT$_{2A}$ receptor/PDZ protein interactions reveals 5-HT$_{2A}$ receptor-operated Ca^{2+} responses in neurons

We next examined the effect of inhibition by the TAT-2ASCV peptide of 5-HT$_{2A}$ receptor/PDZ protein interactions on receptor-mediated signaling in cultured neurons. Application of 5-HT (up to 10 µmol/l) did not induce any detectable increase in cytosolic Ca^{2+} concentration, as assessed by single-cell Fura-2 Ca^{2+} imaging, in most of tested neurons (**Figure 3a,d**). In contrast, following TAT-2ASCV pretreatment (1 µmol/l, 1 hour), 5-HT elicited a clear elevation of cytosolic Ca^{2+} concentration in a subpopulation of neurons (**Figure 3b,d**). Efficient transduction of the peptide into all neurons of the culture was assessed by fluorescent labeling of cell bodies and processes of neurons exposed for 1 hour to the MTS-4-TAT-2ASCV peptide (**Supplementary Figure S4**). Acute application of M100907 (1 µmol/l) blocked the elevation by 5-HT of cytosolic Ca^{2+} levels in neurons pretreated with TAT-2ASCV, indicating the involvement of 5-HT$_{2A}$ receptors (**Figure 3c**). Furthermore, when neurons were pretreated with TAT-2ASCA, no induction of Ca^{2+} responses to 5-HT was detected (**Figure 3d**). These experiments indicate that disruption of 5-HT$_{2A}$ receptor/PDZ protein interactions increases receptor responsiveness in neurons.

We then examined the effect of short hairpin RNA (shRNA) knockdown of endogenous PSD-95, a PDZ partner of spinal
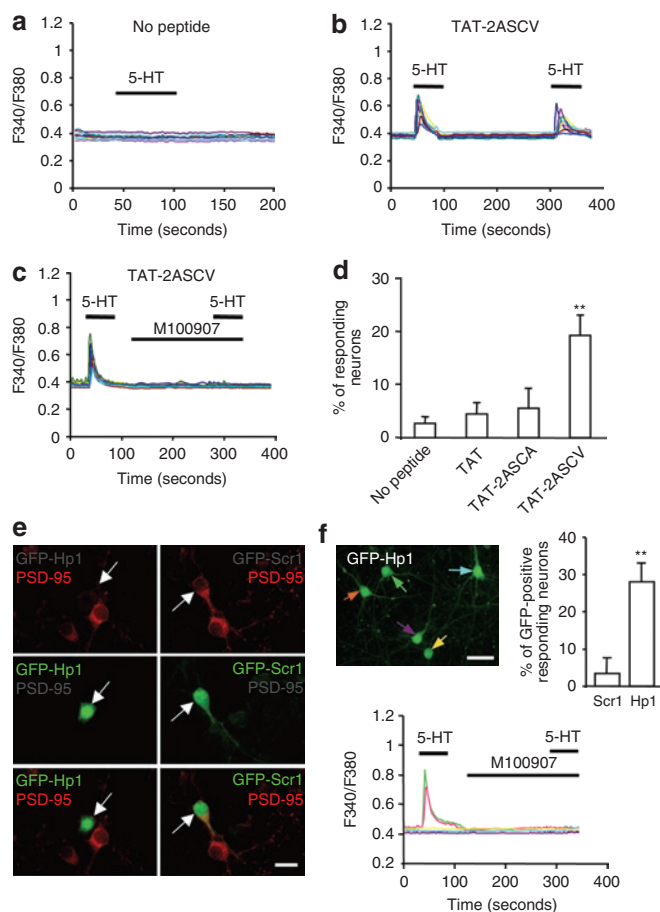

Figure 3 Effects of disruption of 5-HT$_{2A}$ receptor/PDZ protein interactions and of PSD-95 knockdown on receptor signaling in neurons. (**a–c**) Recordings of variations in intracellular Ca^{2+} levels in response to a 10 µmol/l 5-HT challenge in cultured neurons pretreated or not with either TAT-2ASCV (1 µmol/l) for 1 hour. To demonstrate that the 5-HT-evoked Ca^{2+} responses revealed by TAT-2ASCV pretreatment were indeed mediated by 5-HT$_{2A}$ receptors, a second 5-HT challenge was performed in the absence or presence of M100907 (1 µmol/l) after a 3-minute washout period (**c**). Only traces of cells exhibiting Ca^{2+} increase in response to 5-HT are illustrated in **b** and **c**. (**d**) Percentage of neurons in which a 5-HT challenge induced an increase in cytosolic Ca^{2+} concentration. Data are means ± SEM of values obtained in three independent experiments performed on different sets of cultured neurons (four fields containing at least 80 neurons counted per experiment). **$P < 0.01$ versus untreated neurons. (**e**) Neurons were infected with lentiviruses expressing either a GFP-tagged shRNA against PSD-95 (GFP-Hp1) or a GFP-tagged scrambled shRNA (GFP-Scr1) as control. GFP-Hp1-infected neurons exhibited reduced PSD-95 immunoreactivity, compared to GFP-Scr1-infected neurons. Bar = 20 µmol/l. (**f**) Recordings of variations in intracellular Ca^{2+} concentration (bottom) in GFP-Hp1-infected neurons of a representative field (top). Bar: 50 µmol/l. Right: data are means ± SEM of values obtained in three independent experiments performed on different cultures (at least 120 infected neurons counted per condition). **$P < 0.01$ versus Scr1-infected neurons. GFP, green fluorescent protein; 5-HT, 5-hydroxytryptamine; PSD-95, postsynaptic density protein-95; shRNA, short hairpin RNA.

5-HT$_{2A}$ receptors upregulated in diabetic rats. 5-HT elicited cytosolic Ca^{2+} elevation (blocked by M100907) in ≈25% of PSD-95 shRNA (Hp1)-expressing neurons ($n = 120$), which exhibited reduced PSD-95 immunoreactivity in somata and dendrites, but not in neurons expressing scrambled shRNA (Scr1, **Figure 3e,f**).
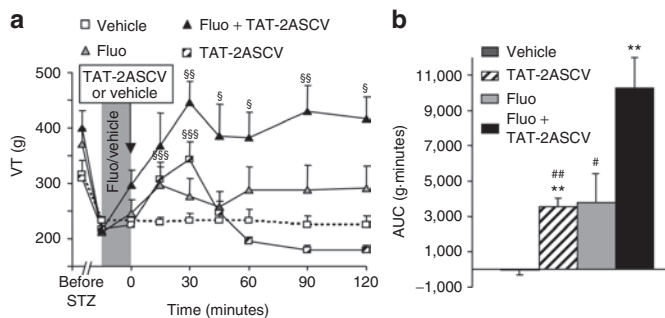
Figure 4 Disruption of 5-HT$_{2A}$ receptor/PDZ protein interactions enhances SSRI-induced antihyperalgesia. (a) Diabetic hyperalgesic rats (six rats/group) received five injections (performed at 12-hour intervals) of either fluoxetine (Fluo, 10 mg/kg, i.p.) or vehicle (hydroxy-propyl-methyl-cellulose, HPMC, 5 ml/kg, i.p.). They were then injected intrathecally with either NaCl (10 µl/rat, vehicle) or the TAT-2ASCV peptide (30 ng/rat). $^{\S}P < 0.05$, $^{\S\S}P < 0.01$, $^{\S\S\S}P < 0.001$ compared with values measured before the fluoxetine/vehicle treatment. **(b)** Area under the time-course (0–60 minute) curve (AUC) of vocalization threshold variations. $^{**}P < 0.01$ versus vehicle; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus Fluo + TAT-2ASCV. HPMC, hydroxy-propyl-methyl-cellulose; i.p., intraperitoneal; SSRI, selective serotonin reuptake inhibitor; STZ, streptozocin; VT, vocalization thresholds.

Thus, PSD-95 knockdown mostly reproduced the effect of TAT-2ASCV, underscoring the critical role of this MAGUK in the regulation of 5-HT$_{2A}$ receptor functional status.

Disruption of 5-HT$_{2A}$ receptor/PDZ protein interactions enhances SSRI-induced antihyperalgesia

In line with the role of 5-HT$_{2A}$ receptor in SSRI-induced anti-allodynic and antihyperalgesic effects in several neuropathic pain models[5,6] and the enhanced responsiveness of 5-HT$_{2A}$ receptor upon disconnection from its PDZ partners, we examined whether disrupting interactions between 5-HT$_{2A}$ receptors and PDZ proteins by the TAT-2ASCV peptide would also increase the effectiveness of the SSRI fluoxetine in diabetic hyperalgesic rats. Repeated administration of fluoxetine (10 mg/kg, 5 intraperitoneal (i.p.) injections performed every half-life time, *i.e.*, at 12-hour intervals) slightly but not significantly increased VT in diabetic hyperalgesic rats at the different times tested (**Figure 4a**), consistent with the weak effectiveness of SSRIs in neuropathic pain.[23] Nonetheless, a significant antihyperalgesic effect of fluoxetine was detected by measuring AUCs of vocalization threshold variations (**Figure 4b**). Administration of fluoxetine combined with an administration of TAT-2ASCV (30 ng/rat, single i.t. injection) 6 hours after the last fluoxetine injection produced a marked antihyperalgesic effect, which was higher and more prolonged than those induced by fluoxetine or the peptide alone (**Figure 4a,b**).

DISCUSSION

In the present study, we showed that stimulation of spinal 5-HT$_{2A}$ receptors induced a clear antinociceptive effect in healthy rats. This antinociceptive action was mediated by recruitment of postsynaptic receptors located on GABAergic neurons, as it was totally abolished by the GABA-A receptor antagonist bicuculline. GABA release from spinal GABAergic interneurons upon 5-HT$_{2A}$ receptor activation, in turn inhibits nociceptive projection

neurons innervating supraspinal areas.[1] In contrast, stimulation of 5-HT$_{2A}$ receptors did not produce analgesic effects in diabetic neuropathic rats. Several lines of evidence indicate that the loss of 5-HT$_{2A}$ receptor-mediated analgesia in diabetic neuropathy result from an alteration of 5-HT$_{2A}$ receptor functional activity upon association with PDZ proteins and not from receptor downregulation: (i) the total number of 5-HT$_{2A}$ receptor binding sites was not decreased in spinal cord of neuropathic rats; (ii) overall disruption of interactions between spinal 5-HT$_{2A}$ receptor and associated PDZ proteins by intrathecal injection of a cell-penetrating peptidyl mimetic of receptor C-terminus significantly inhibited mechanical hyperalgesia and suppressed the spontaneous temperature preference behavior induced by diabetic neuropathy; (iii) this antihyperalgesic action resulted from activation of 5-HT$_{2A}$ receptors by endogenous 5-HT, as it was suppressed by co-injection of the 5-HT$_{2A}$ antagonist M100907; and (iv) treatment of cultured neurons with the peptide revealed functional 5-HT$_{2A}$ receptor-operated Ca^{2+} responses, indicating that association of receptor with PDZ proteins might be responsible for a tonic inhibition of receptor signaling. It is noteworthy that the interfering peptide selectively suppressed neuropathic pain in diabetic rats without affecting acute mechanical or thermal nociception in healthy animals, indicating a minor influence of interactions between spinal 5-HT$_{2A}$ receptors and PDZ proteins in acute pain processing and/or an absence of a serotonergic tone in spinal cord of healthy rats. This result is in line with the lack of intrinsic effect of the 5-HT$_{2A}$ antagonist observed in the present study and with the lack of change in pain-induced behavior shown by others in mice lacking 5-HT receptors in conditions of acute pain.[24]

We identified four major PDZ proteins interacting with 5-HT$_{2A}$ receptor in the spinal cord, which are potentially involved in alteration of receptor analgesic function in diabetic neuropathy. These included three proteins previously identified as PDZ partners of the receptor in the brain (PSD-95, SAP97, and MAGI2) and a novel partner (MPP2) only detected in spinal cord. In contrast, we did not detect two PDZ proteins previously identified as receptor interacting proteins in brain, MPP3, and channel interacting PDZ protein. This different profile of PDZ partners likely reflects their different expression levels in brain and spinal cord. PSD-95 has previously been implicated in the development and the maintenance of neuropathic pain in rats,[14,15,18,19,25] neuropathic sensitization of behavioral reflexes following sciatic nerve injury in mice[17] and NMDA-induced thermal hyperalgesia in mice.[25] Our results suggest that PSD-95 might contribute, at least in part, to alteration of 5-HT$_{2A}$ receptor-induced analgesia in painful diabetic neuropathy. First, expression of PSD-95 was significantly increased in spinal cord dorsal horn of diabetic hyperalgesic rats, compared with healthy rats. This increased expression of PSD-95 might lead to a disorganization of the PDZ protein network interacting with spinal 5-HT$_{2A}$ receptors and an increase in the fraction of receptors bound to this MAGUK in diabetic neuropathic rats. Second, knockdown of PSD-95 in neurons using shRNA increased receptor responsiveness, thereby reproducing the effect of the TAT-2ASCV peptide. An increase in the receptor fraction associated with this MAGUK, which inhibits receptor-mediated signal transduction, might thus account for the resistance of diabetic rats to analgesic effects of 5-HT$_{2A}$ receptor agonists.

A role of PSD-95 in hallucinogen and antipsychotic drug actions at 5-HT$_{2A}$ receptors has recently been described.[26] The specific influence of the interaction of 5-HT$_{2A}$ receptors with PSD-95 in the resistance of diabetic hyperalgesic rats to 5-HT$_{2A}$ receptor-mediated analgesic effects could not be definitely established by means of spinal PSD-95 knockdown using shRNA. Indeed, knockdown of PSD-95 was previously shown to strongly affect NMDA receptor-dependent hyperalgesia,[16] precluding discrimination between NMDA receptor- and 5-HT$_{2A}$ receptor-dependent effects on nociceptive transmission. This contrasts with the peptidyl mimetic approach used in the present report, which selectively impaired 5-HT$_{2A}$ receptor/PSD-95 interaction without affecting association of this MAGUK with NMDA receptor and thereby did not alter NMDA receptor-dependent modulation of pain hypersensitivity. Alternative strategies based on disruption of protein–protein interactions within the NMDA receptor complex have recently been proposed to suppress acute and chronic pain hypersensitivity depending on NMDA receptors.[21,25,27] These studies together with the present findings point out the relevance of strategies blocking protein–protein interactions to manage chronic pain, with presumably less pronounced side effects than classical approaches based on receptor agonists or antagonists.

Due to the critical role of 5-HT$_{2A}$ receptors in SSRI-induced analgesic effects,[5,6] the decreased analgesic function of these receptors in diabetic hyperalgesic rats might be responsible for the low efficacy of SSRIs in painful neuropathy, compared with other antidepressants inhibiting reuptake of norepinephrine as well, such as the tricyclic amitriptyline.[23] Consistent with this hypothesis, disconnection of 5-HT$_{2A}$ receptors from their PDZ partners strongly enhanced the antihyperalgesic action of fluoxetine in diabetic neuropathic rats. Moreover, a prolonged analgesia was observed in animals co-injected with fluoxetine and the TAT-2ASCV peptide. The low efficacy of SSRIs to relieve neuropathic pain lead clinicians to prescribe more efficacious but less tolerated compounds, such as amitriptyline, the gold standard of analgesic antidepressants.[23] However, a substantial percentage of patients does not favorably respond to this compound,[28] which might reflect an antagonistic activity at 5-HT$_{2A}$ receptor.[29] Our data show that SSRIs become effective against neuropathic pain providing that spinal 5-HT$_{2A}$ receptors are disconnected from their associated PDZ proteins. Targeting interactions between 5-HT$_{2A}$ receptors and associated PDZ proteins, which is efficient against both mechanical and thermal hyperalgesia, two positive sensory signs frequently observed in neuropathic pain,[30] could be an attractive strategy to increase the effectiveness of SSRIs in this context.

MATERIALS AND METHODS

Chemicals. Streptozocin (STZ, Zanosar) was purchased from Pfizer (Amboise, France), insulin (Lantus) from Sanofi-Aventis (Paris, France), α-methyl-5-HT maleate, SB242084, bicuculline and cocaine from Sigma (St Quentin Fallavier, France), fluoxetine from RPG Laboratory (Paris, France) and diazepam (Valium) from Roche (Neuilly-sur-Seine, France). M100907 was generously provided by Dr Clotilde Mannoury la Cour (Servier Pharmaceuticals, Croissy sur Seine, France). Drugs were dissolved in physiological saline (NaCl, 0.9%), except M100907, SB242084, and bicuculline, dissolved in 50, 50, and 30% dimethyl sulfoxide, respectively, fluoxetine, dissolved in hydroxy-propyl-methyl-cellulose (Colorcon,

Orpington, England), STZ, dissolved in distilled water and diazepam dissolved in ethanol.

Peptides, shRNAs, and antibodies. Synthetic peptides (>95% purity) were purchased from Eurogentec (Seraing, Belgium). Peptide sequences were as follows: 5-HT$_{2ASCV}$Ct, TDNIETVNEKVSCV; 5-HT$_{2ASCA}$Ct, TDNIETVNEKVSCA; TAT-2ASCV, YGRKKRRQRRRTVNEKVSCV; TAT-2ASCA, YGRKKRRQRRRTVNEKVSCA. The TAT-2ASCV peptide and the corresponding peptide lacking the TAT sequence (TAT-empty-2ASCV, TVNEKVSCV) were synthesized with an additional *N*-terminal cysteinyl residue and conjugated to 2-[(5-fluoresceinyl)aminocarbonyl] ethyl MTS (MTS-4-fluorescein; Toronto Research; North York, Ontario, Canada) to monitor peptide transduction into spinal cord neurons following intrathecal delivery by fluorescence microscopy.[31]

Constructs expressing short hairpin RNA (shRNA) against PSD-95 (Hp1) or scrambled shRNA (Scr1) under control of U6 promoter were modified from ref. 3 for lentiviral coexpression with green fluorescent protein under control of ubiquitin promoter.[32] Lentiviral production has been described elsewhere.[32,33] Cultured neurons were infected at 3 days *in vitro* and experiments were performed at 13 days *in vitro*.

The mouse monoclonal anti-5-HT$_{2A}$ receptor antibody was obtained from BD Biosciences (San Jose, CA), the rabbit polyclonal anti-5-HT$_{2A}$ receptor antibody from Abcam (Cambridge, UK), the mouse anti-PSD-95 IgG$_{2A}$ (clone K28/43) from Upstate (Lake Placid, NY), the mouse anti-SAP97 IgG$_1$ from StressGen Bioreagents (Victoria, British Columbia, Canada), the mouse monoclonal anti-NR1 antibody from Zymed Laboratories (San Francisco, CA), the rabbit polyclonal anti-NR1 antibody from Upstate and the mouse anti-pan-actin monoclonal antibody from NeoMarkers (Fremont, CA).

Animals and behavioral testing. All experiments were performed on adult male Sprague–Dawley rats (200–250 g) (Charles River, Cléon, France) in accordance with the IAPS guidelines[34] and were approved by the local ethics committee of Auvergne (agreement no. CE3-06). Diabetes was induced by an i.p. injection of STZ (72 mg/kg). Healthy rats only received distilled water (5 ml/kg, i.p.). Diabetic rats (blood glucose level >14 mmol/l) received every other day a subcutaneous insulin injection (2 IU/rat), from 7 days after STZ injection. Diabetic rats were previously found to display abnormal pain-associated behaviors, measured as exaggerated responses to painful stimuli (hyperalgesia).[35]

Hypersensitivity to mechanical stimuli was determined by applying an increasing pressure to the left hind paw until a squeak was elicited (vocalization threshold expressed in grams, maximal pressure applied was 550 g). Data are expressed as mean ± SE of the mean (SEM) of the VT (g). To investigate global effects, areas under the time-course curves (AUC) of the vocalization threshold variations between 0 and 60 minutes were calculated using the trapezoidal rule as followed: $\Sigma[(VT_{time\ X} - VT_{time\ 0}) \cdot (time\ X - time\ X{-}1)]$. Diabetic rats were considered hyperalgesic if the VT were reduced to at least 20%, compared with VT measured before STZ treatment. Hyperalgesia to thermal stimuli was assessed using the two-temperature choice test.[20] Rats were freely moving on two adjoining plates set at different temperatures: 25 °C (thermoneutral plate) and the other one at 45 °C (nociceptive plate). They were free to choose between occupying the hot surface versus staying on the surface at neutral temperature. For this test, data are expressed as time spent on the hot surface (seconds). When both plates were set to 25 °C, rats spent about 50% of the time *i.e.*, 90 seconds on each plate. When diabetic rats were presented to a choice of occupying the hot surface versus the neutral surface, a 20% decrease in the time spent on the hot surface was measured compared with healthy rats, indicating thermal hyperalgesia. Only hyperalgesic diabetic rats were included in the study. Different rats were used for the different behavioral testings.

Hyperalgesic diabetic and healthy rats were injected i.t. with drugs 3 weeks after the STZ/vehicle treatment. In experiments using α-methyl-5-HT or

cell-penetrating peptides, the VT to paw-pressure were measured up to 120 minutes after the injection and the time spent on each surface was measured at 30 minutes. In experiments using fluoxetine, diabetic hyperalgesic rats received five fluoxetine injections (10 mg/kg i.p. each, one injection every 12 hours, 8:00 AM and 8:00 PM). Six hours after the last fluoxetine injection, rats were injected with the TAT-2ASCV peptide (30 ng/rat, i.t.) or the vehicle and submitted to the paw-pressure test for 120 minutes.

Motor function, muscular tone, and coordination were estimated by measuring spontaneous locomotor activity, grip strength response, and righting reflex response, respectively. To determine spontaneous locomotor activity, rats were placed in a rectangular cage (45 × 30 × 20 cm) and allowed to investigate the cage for 3 minutes, during which the total number of movements were recorded (Actisystem, Penlab, Apelex). The grip strength was measured by placing the rat forelimbs on a dynamometer (Bioseb, Chaville, France) and pulling on its tail until the rat released the grill. At that time, the score (expressed in arbitrary units) is read on the strain gauge. For determination of the righting reflex response, rats were held on their back on a padded flat surface and then released. The righting reflex was considered positive (quoted 1) if the rats lying on their back were able to right themselves on all four limbs within 1 second. Three consecutive trials were performed (maximal score: 3). These parameters were determined after injection of either vehicle (10 µl/rat, i.t.), TAT-2ASCV (30 ng/rat, i.t.), cocaine (1 mg/kg, intravenously), or diazepam (2,5 mg/kg, i.p.).

All the *in vivo* experiments were performed blind by a single experimenter using a parallel group design. Treatments were randomized.

Preparation of spinal cord extracts. Hyperalgesic diabetic and healthy rats were sacrificed by decapitation. Lumbar enlargements of the spinal cord dorsal horn were rapidly removed and homogenized on ice with lysis buffer containing Tris-HCl, 50 mmol/l, pH 7.4; EDTA, 0.5 mmol/l; 3-[(3-cholamidopropyl) dimethylammonio]propanesulfonate (1.3%, wt/vol), and a protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany). Then, samples were centrifuged for 1 hour at 10,000g and the supernatants containing solubilized proteins were collected.

Peptide affinity chromatography. Solubilized proteins (5 mg/condition) were incubated in the presence of either a peptide encompassing the 14 C-terminal residues of the mouse and rat 5-HT$_{2A}$ receptor (5-HT$_{2ASCV}$Ct) or a modified peptide in which the C-terminal valine residue was substituted into an alanine (5-HT$_{2ASCA}$Ct), immobilized onto activated CH-sepharose 4B (GE Healthcare, Uppsala, Sweden), as previously described.[13] Samples were washed five times with lysis buffer supplemented with 5 mol/l NaCl and once with lysis buffer without NaCl. Proteins retained by affinity were eluted off with either sodium dodecyl sulfate (SDS) sample buffer (Tris-HCl, 50 mmol/l, pH 6.8; SDS, 2%; glycerol 30%; dithiothreitol 100 mmol/l, and bromophenol blue) for SDS-polyacrylamide gel electrophoresis and immunoblotting or isoelectrofocusing medium containing urea (7 mol/l), thiourea (2 mol/l), 3-[(3-cholamidopropyl) dimethylammonio]propanesulfonate (4%), ampholines (preblended, pI 3.5–9.5, 8 mg/ml; GE Healthcare), dithiothreitol (100 mmol/l), tergitol NP7 (0.2%) and traces of bromophenol blue for 2-D electrophoresis.

2-D electrophoresis and 2-D gel protein pattern analysis. Proteins were first separated according to their isoelectric point along linear immobilized pH-gradient strips (pH 3–10, 18-cm long; GE Healthcare). Sample loading for the first dimension was performed by passive in-gel reswelling. After the first dimension, the immobilized pH-gradient strips were equilibrated for 10 minutes in a buffer containing urea (6 mol/l), Tris-HCl (50 mmol/l, pH 6.8), glycerol (30%), SDS (2%), dithiothreitol (10 mg/ml), and bromophenol blue and then for 15 minutes in the same buffer containing 15 mg/ml iodoacetamide instead of dithiothreitol. For the second dimension, the strips were loaded onto vertical 12.5% SDS-polyacrylamide gel electrophoresis. The gels were stained with silver as previously described.[36]

Gels to be compared were always processed and stained in parallel. Gels were scanned using a computer-assisted densitometer. Spot detection, gel alignment, and spot quantification were performed using the Image Master Platinium/Melanie 5 software (GE Healthcare). Quantification of proteins was expressed as volumes of spots. To correct the variability resulting from silver staining, results were expressed as relative volumes of total spots in each gel.

Matrix assisted laser desorption/ionization time-of-flight mass spectrometry and protein identification. Proteins of interest were excised and digested in gel using trypsin (Gold; Promega, charbonniéres-les-Bains, France), as previously described.[13] Digest products were completely dehydrated in a vacuum centrifuge and resuspended in 10 µl formic acid (2%), desalted using Zip Tips C18 (Millipore, Molsheim, France), eluted with 10 µl acetonitrile-trifluoroacetic acid (50–0.1%) and concentrated to 2 µl. Aliquots (0.5 µl) were mixed with the same volume of α-cyano-4-hydroxytrans-cinnamic acid (Sigma; 10 mg/ml in acetonitrile-trifluoroacetic acid, 50–0.1%) and loaded on the target of an Ultraflex matrix assisted laser desorption/ionization time-of-flight mass spectrometer (Bruker Daltonik, Bremen, Germany). Analyses were performed in reflectron mode with an accelerating voltage of 25 kV and a delayed extraction of 50 ns. Spectra were analyzed using the FlexAnalysis software (version 2.4; Bruker Daltonik) and autoproteolysis products of trypsin (molecular weight: 842.51, 1,045.56, 2,211.10, 2,383.90) were used as internal calibrates. Identification of proteins was performed using the Mascot software (version 2.1; Matrix science, London, UK) against the Swiss-Prot or TrEMBL databases. The following parameters were used for database interrogation: mass tolerance of 50 p.p.m. (even if the mass accuracy of our analyses was usually better than 20 p.p.m.); fixed chemical modification, carbamidomethylation of cysteins; variable chemical modification, oxidation of methionines; matching peptides with one missed cleavage accepted only when they included two consecutive basic residues or when arginine or lysine residues were followed by one or several acidic residues inside the peptide aminoacid sequence. Mascot scores >63 and 68 were considered as significant ($P < 0.01$) for Swiss-Prot and TrEMBL database interrogation, respectively.

Co-immunoprecipitation. 3-[(3-Cholamidopropyl) dimethylammonio]propanesulfonate-soluble dorsal spinal cord protein extracts (5 mg/experiment) were incubated overnight at 4 °C with the rabbit polyclonal anti-5-HT$_{2A}$ receptor antibody or the monoclonal anti-NR1 antibody (5 µg). Samples were then incubated for 1 hour at 4 °C with 50 µl of immobilized protein A/G beads (Thermoscientific, Courtabeuf, France). After five washes with lysis buffer, immunoprecipitated proteins were eluted in SDS sample buffer, resolved by SDS-polyacrylamide gel electrophoresis and detected by western blotting.

Western blotting. Proteins resolved on polyacrylamide gels were transferred electrophoretically to nitrocellulose membranes (Hybond-C; GE Healthcare) and stained with Ponceau red, to assess transfer of equal amounts of proteins in each lane. Membranes were blocked with 5% nonfat dry milk diluted in Tris-buffered saline Tween-20 and incubated successively with primary antibodies (anti-5-HT$_{2A}$ receptor mouse IgG$_1$, 1:500, anti-PSD-95 mouse IgG$_{2A}$, 1:50,000, anti-SAP97 mouse IgG$_1$, 1:500, rabbit anti-NR1, 1:500, mouse anti-pan-actin, 1:2,000) overnight at 4 °C and with a horse-radish peroxidase-conjugated anti-mouse antibody (1:3,000; GE Healthcare) for 1 hour at room temperature. Immunoreactivity was detected with an enhanced chemiluminescence method (ECL detection reagent; GE Healthcare).

[³H]-ketanserin saturation analysis. Lumbar dorsal spinal cords from healthy and diabetic hyperalgesic rats were homogenized in 50 mmol/l Tris-HCl buffer, pH 7.4, containing 0.5 mmol/l EDTA, 10 mmol/l MgSO$_4$, and a cocktail of protease inhibitors (Roche Diagnostics, Basel, Switzerland) and homogenates were centrifuged for 5 minutes at 1,000g. Supernatants were then centrifuged at 20,000g for 20 minutes. The

pellets (membrane fraction) were resuspended in the binding buffer containing 50 mmol/l Tris-HCl, pH 7.4, 0.5 mmol/l EDTA, 10 mmol/l MgSO$_4$, 10 µmol/l pargyline, and 0.1% (wt/vol) ascorbic acid. Membranes (100 µg/assay) were incubated with 0.5–15 nmol/l [^{3}H]-ketanserin (67 Ci/mmol; Perkin Elmer, Courtaboeuf, France) for 2 hours at 22 °C in a total volume of 300 µl. Prazosin (30 nmol/l) was included in all assays to preclude binding to α$_1$ receptors. Nonspecific binding was determined in the presence of 50 µmol/l mianserin. The reaction was terminated by rapid filtration over GF-B filters presoaked with 20 mg/ml bovine serum albumin. Radioactivity retained on the filters was determined by scintillation counting. B_{max} and Kd were determined by Scatchard analysis of saturation data.

Neuronal cultures and Ca^{2+} imaging. Primary cultures of cortical neurons were prepared as previously described.[31] Briefly, cells from the cerebral cortex of 17-day-old Swiss mouse embryos were plated in serum-free medium on Lab-Tek II chamber slides (1 × 10^6 cells/well; Nalge Nunc International, Naperville, IL), coated successively with poly-L-ornithine (moleculat weight = 40,000; 15 µg/ml) and 10% fetal calf serum + 1 µg/ml laminin (Sigma). The culture medium included a 1:1 mixture of Dulbecco's modified Eagle's medium and F-12 nutrient supplemented with 33 mmol/l glucose, 2 mmol/l glutamine, 13 mmol/l NaHCO$_3$, 5 mmol/l HEPES buffer, pH 7.4, 5 IU/ml-5 mg/ml) penicillin–streptomycin, and a mixture of salt and hormones containing 100 µg/ml transferrin, 25 µg/ml insulin, 20 nmol/l progesterone, 60 nmol/l putrescine, and 30 nmol/l Na$_2$SeO$_3$. Cultures were maintained for 7 days at 37 °C in a humidified atmosphere in 5% CO$_2$, 95% air. At this stage, cultures were shown to contain at least 95% of neurons.[31]

Cells were loaded with Fura-2 acetoxymethyl ester (FluoProbes, Interchim, Montluçon, France) at a final concentration of 12.5 µmol/l for 30 minutes at 37 °C in Locke's solution containing 140 mmol/l NaCl, 1.2 mmol/l KH$_2$PO$_4$, 5 mmol/l KCl, 1.2 mmol/l MgSO$_4$, 10 mmol/l HEPES, 1.8 mmol/l CaCl$_2$, and 10 mmol/l glucose. After loading, cells were rinsed twice and incubated for 30 minutes in dye-free Locke's buffer. Lab-Teks were then placed on the stage of an IX70 Olympus microscope (Olympus, Tokyo, Japan) and continuously superfused with Locke's solution. Imaging of intracellular calcium changes in individual cells treated with agonist was accomplished by ratiometric imaging of Fura-2 fluorescence at 340- and 380-nm excitation using the MetaFluor Imaging system (Molecular Devices, Sunnyvale, CA). Fluorescence was excited by illumination via a 20× water immersion objective with rapid light wavelength switching provided by a DG4 filter wheel (Sutter Instrument, Novato, CA) and detected by a charge-coupled device camera under the control of MetaFluor software. Before agonist stimulation, images were obtained for 30 seconds to establish a stable baseline Ca^{2+} measurement. Our standard protocol consisted of two sequential 5-HT applications, separated by 3-minute washouts to allow complete refilling of Ca^{2+} stores following the first 5-HT challenge. Individual Ca^{2+} traces in the figures are representative responses for a given field of cells.

Statistics. A two-ways variance analysis (analysis of variance, repeated measures) followed by Bonferroni's test was performed to analyze the evolution of VT. An unpaired Student's *t*-test was used to compare the effect of different treatments or to compare AUC between diabetic hyperalgesic rats and healthy rats.

Immunoblots were quantified by densitometric analysis, using the Phoretix Software and normalized against actin. Difference between diabetic hyperalgesic rats and healthy rats was analyzed by an unpaired Student's *t*-test.

The statistics software used was InStat 3.00 (GraphPad, San Diego, CA). The significance level was set at $P < 0.05$.

SUPPLEMENTARY MATERIAL
Figure S1. Recruitment of spinal cord PDZ proteins by the 5-HT$_{2A}$ receptor C-terminus.

Figure S2. Inhibition of 5-HT$_{2A}$ receptor/PDZ protein interactions by a cell penetrating peptidyl mimetic of the 5-HT$_{2A}$ receptor C-terminus.
Figure S3. Lack of effect of the TAT-2ASCV peptide on NMDA receptor/PSD-95 interaction, NMDA-induced mechanical hyperalgesia and motor function in healthy rats.
Figure S4. Efficient penetration of the TAT-2ASCV peptide in cultured neurons.
Table S1. Results of MALDI-TOF MS analyses.

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