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REVIEW ARTICLE

Serotonergic Modulation of Nociceptive Circuits in Spinal Cord Dorsal Horn

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Abstract: ***Background*:** Despite the extensive number of studies performed in the last 50 years, aimed at describing the role of serotonin and its receptors in pain modulation at the spinal cord level, several aspects are still not entirely understood. The interpretation of these results is often complicated by the use of different pain models and animal species, together with the lack of highly selective agonists and antagonists binding to serotonin receptors.

***Method*:** In this review, a search has been conducted on studies investigating the modulatory action exerted by serotonin on specific neurons and circuits in the spinal cord dorsal horn. Particular attention has been paid to studies employing electrophysiological techniques, both *in vivo* and *in vitro*.

***Conclusion*:** The effects of serotonin on pain transmission in dorsal horn depend on several factors, including the type of receptors activated and the populations of neurons involved. Recently, studies performed by activating and/or recording from identified neurons have importantly contributed to the understanding of serotonergic modulation on dorsal horn circuits.

Keywords: Pain, serotonin receptors, dorsal horn, descending modulation, electrophysiology, synaptic transmission.

1. INTRODUCTION

Serotonergic fibers derive from rostral ventromedial medulla (RVM) and caudal pons, including nucleus raphe magnus (NRM), nucleus paragigantocellularis (PG) and the ventral portions of the nucleus gigantocellularis [1, 2]. They represent about 20% of fibers descending from RVM, while the majority of fibers derive from GABA/glycinergic neurons [3]. Serotonergic fibers descend through the dorsolateral funiculus and terminate mainly in the superficial dorsal horn (DH), where the first central synaptic relay of nociceptive signals generated at peripheral nociceptors is located. A recent study has shown that the median NRM neurons project preferentially into the deep laminae V-VI of the DH whereas the lateral PG neurons send projections exclusively into the superficial laminae I-II, supporting the idea that serotonergic neurons might be differently implicated in pain signaling modulations depending on their location in the NRM *versus* the PG [4]. The majority of serotonergic fibers form non-synaptic varicosities in the vicinity of DH neurons and astrocytes, while only 20% of terminals contribute to classical synaptic contacts [5, 6]. This organization suggests that most serotonergic transmission occurs through volume transmission [7], involving both neurons and astrocytes. Tonic release of serotonin (5-HT) in the DH could occur, since some NRM neurons fire spontaneously [8].

So far, 14 types of serotonergic receptors (5-HTRs) have been characterized and most of them are expressed on nociceptors and/or DH neurons in the rodent and human spinal cord. I will focus on 5-HT_1 (A and B), 5-HT_2 (A and C), 5-HT_3, and 5-HT_7 receptors, whose properties have been more extensively investigated. As I will describe in sections 3-6, 5-HT can exert both pro-nociceptive and analgesic effects by activating specific types of 5-HT receptors in the spinal cord DH, in different pain conditions. Consistently with this bidirectional action of 5-HT, pure serotonergic drugs, such as selective 5-HT reuptake inhibitors (SSRIs) that increase only 5-HT levels, have low efficacy in the treatment of chronic pain. On the other hand, more balanced 5-HT/noradrenaline reuptake inhibitors, such as duloxetine and venlafaxine, have been proved effective as first-line drugs for the treatment of neuropathic pain [9, 10].

2. OVERVIEW ON SEROTONERGIC RECEPTORS LOCATED IN DORSAL HORN

5-HT_1Rs constitute the main type of 5-HTR in the spinal cord, accounting for about 25% of the total population. They are negatively coupled to adenylyl-cyclase, causing the opening of potassium channels and/or the closing of calcium channels, and inducing neuron hyperpolarization. Autoradiography studies have shown that **5-HT_{1A}Rs** are widely

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expressed in the rat spinal cord DH, particularly in superficial laminae, with a rostrocaudal gradient [11, 12]. High levels of 5-HT_{1A}Rs have also been detected in the superficial DH of the human spinal cord [13]. *In situ* hybridization, single-cell PCR and double immunofluorescence staining studies have confirmed the presence of 5-HT_1Rs in the rodent DH. In GAD67-GFP knock-in mice, 5-HT_{1A}Rs are located on about 50% of GABAergic interneurons and their expression on GABAergic and enkephalinergic DH interneurons increases following carrageenan-induced peripheral inflammation [14, 15]. The presence of 5-HT_{1A}Rs on primary afferent fibers (PAFs) is still a matter of debate: autoradiography studies report that 5-HT_{1A}R binding to agonists (such as 8-OH-DPAT) in DH decreases after dorsal rhizotomy or neonatal treatment with capsaicin, suggesting the localization of these receptors on nociceptive PAFs [16, 17]. On the other hand, mRNA encoding 5-HT_{1A} has not been detected in significant amounts in the dorsal root ganglia (DRGs) [18-20]. Data from electrophysiological studies would confirm the presence of functional 5-HT_{1A}Rs on PAF terminals in DH (sections 4-5).

5-HT_{1B}Rs are negatively coupled to adenylyl cyclase and their activation leads to the increase of potassium currents. These receptors have been shown in rat DH, particularly in laminae I, III and IV, postsynaptically to serotonergic fibers [11]. Dorsal rhizotomy causes a 20% decrease of 5-HT_{1B} radioligand [125I]GTI binding in DH, suggesting the expression of 5-HT_{1B}Rs also on a subpopulation of PAFs [17]. Accordingly, 5-HT_{1B} mRNA has been detected in the rat lumbar DRGs [19].

5-HT_2Rs are positively coupled to phospholipase C, inhibit potassium currents causing neuron excitation. The main types found in rodent spinal cord are 2A and 2C, mainly located in the sympathetic area and the ventral horn, while a lower expression has been detected in DH [11]. Immunohistochemical studies report the expression of 5-HT_{2A}Rs on small to medium size DRG neurons [21-23], on peptidergic axon terminals in the DH, on DH neurons (mainly on dendrites and cell bodies) [24], and on astrocytes [25]. By performing single-cell RT-PCR on GAD67-GFP knock-in mice, 5-HT_{2A} mRNA has been found in a limited subpopulation of GABAergic interneurons [15]. Low level of 5-HT_{2A} mRNA expression, detected in rat DRGs and DH, is amplified by peripheral inflammation [22]. The 5-HT_{2C} expression has been reported within lumbar DRGs [19], on PAF terminals and in different regions of spinal grey matter, particularly on the neuronal somata and dendrites [26-29].

5-HT_3R, the only ionotropic 5-HTR, is a pentameric channel permeable to cations, causing the depolarization of neurons and the increase of excitability. Spinal 5-HT_3Rs are located on PAF terminals in the DH, as evidenced by the decrease of 5-HT_3 ligand binding after dorsal rhizotomy or neonatal capsacin treatment [17, 30]. Immunohistochemical and *in situ* hybridization studies have reported the expression of 5-HT_3Rs both in DRG and DH neurons [31-33]. Conte *et al.* [34] have shown the presence of 5-HT_3R on excitatory axon terminals in the DH, some of which originate from excitatory interneurons positive to calbindin. Single-cell RT-PCR performed on mouse DH has evidenced that a subpopulation of GABAergic and enkephalinergic neurons expresses 5-HT_3Rs [35, 36]. Furthermore, lamina I neurons expressing NK1 receptors are associated with 5-HT_3 positive axons with a variable density of synaptic contacts [34]. The NK1 positive neurons in the DH include at least two populations of cells: neurons with a large cell body, representing projection neurons, and neurons with a small soma, identified as interneurons [37].

Finally, **5-HT_7R** has been recently identified as a metabotropic receptor positively coupled to adenylyl cyclase, causing the increase of neuronal excitability. 5-HT_7 mRNA has been found in rat and human DRGs [38]. Subsequent studies have shown the protein localization mainly in small-diameter DRG neurons and on unmyelinated and thinly myelinated PAFs, suggesting a predominant expression on nociceptive fibers. In the DH, 5-HT_7Rs are expressed on peptidergic neurons (mainly in superficial DH), GABAergic interneurons (mainly in laminae III-V), and on astrocytes [39-41].

3. BEHAVIOURAL STUDIES ON SEROTONERGIC PAIN MODULATION IN THE SPINAL CORD

The serotonergic descending system is functionally bidirectional, producing either pain inhibition or facilitation, depending on behavioral conditions. Several studies report that direct electrical stimulation of the periaqueductal grey (PAG) or RVM (in particular of NRM) induces both 5-HT release in the spinal cord and anti-nociceptive effects [42-47]. Focal electrical stimulation of the RVM raphe nuclei reticularis gigantocellularis and gigantocellularis pars alpha induces either facilitation or inhibition of the spinal nociceptive tail-flick reflex, depending on the intensity of stimulation: low intensities cause facilitation, while higher intensities produce inhibition [48]. Using pharmacological or genetic models to manipulate the descending serotonergic system, other authors have confirmed the bidirectional modulation exerted by 5-HT on pain. Microinjection of neurotensin in the RVM (activating spinally projecting serotonergic neurons) produces dose-dependent antinociception [49], while optogenetic activation of RVM serotonergic neurons induces pain sensitization [50]. Depleting 5-HT in RVM neurons with regional shRNA interference (RNAi) of tryptophan hydroxylase-2 (the rate-limiting enzyme in the 5-HT synthesis) attenuates formalin-induced spontaneous nocifensive responses and tissue or nerve injury-induced allodynia and hyperalgesia, suggesting a major facilitating role of the RVM under persistent pain conditions [51]. Lmx1b conditional knock-out mice lacking serotonergic neurons show normal thermal and visceral pain responses but are less sensitive to mechanical stimuli and exhibit enhanced inflammatory pain [52]. Persistent inflammatory and neuropathic pain epigenetically depress transcription of *Gad2* (encoding glutamic acid decarboxylase 65, GAD65) in rat NRM [53]. The consequent decrease of GABA synthesis and disinhibition of NRM serotonergic neurons might induce functional changes in serotonergic descending pathways, leading to central sensitization and chronic pain.

The complexity of serotonergic modulation on nociception in the spinal cord derives from several factors, including

the multiplicity of 5-HTRs activated and the different types of neurons involved. Intrathecal (i.t.) injection of 5-HT in rats produces an analgesic effect by increasing the tail-flick response latency and behavioral responses to substance P, as reported by early studies [54-57]. Administration of **5-HT_{1A}** agonists to the spinal cord produces both pro- and anti-nociceptive effects in adult rodents. Some authors have reported that i.t. or intraspinal administration of the 5-HT_{1A} agonist 8-OH-DPAT decreases the pain behavioral response [57-59]. Other studies have described a more complex action of 5-HT_{1A}R agonists in the DH: injection of F13640 or i.t administration 8-OH-DPAT decrease vocalization threshold to paw pressure in rats, while inhibiting pain behavior in the formalin test [60-62]. Similarly, i.t. administration of 8-OH-DPAT decreases mechanical allodynia in carrageenan-induced inflammation [63]. It has been proposed that at mild noxious intensities, 5-HT_{1A} agonism promotes nociception, while it has an anti-nociceptive effect at stronger nociceptive stimulations. Accordingly, spinal administration of F13640 increases formalin-induced spinal c-Fos protein expression in rats, while administration concomitant with strong noxious stimulation reduces c-Fos expression [64]. These bidirectional effects could be partly explained by different targets of 5-HT_{1A} mediated inhibition: pro-nociceptive effects in acute pain could be due to the inhibition of GABAergic interneurons, while anti-nociception could be obtained by depressing the activity of projection neurons during persistent pain [62]. The low selectivity of the 5-HT_{1A} agonists used in these studies (8-OH-DPAT, for example, activates also 5-HT_7Rs) further complicates the interpretation of these results. **5-HT_{1B}Rs** seem to exert a prevalent anti-nociceptive effect at the spinal level. Intrathecal administration of the 5-HT_{1B} agonist RU 24969 inhibits nociceptive tail-flick reflex in mice [58] and 5-HT_{1B} knock-out mice exhibit higher thermal and formalin sensitivity (similarly to 5-HT_{1A} knock-out mice) [65]. Mechanisms involving direct inhibition of projection neurons and suppression of glutamate and peptide release from PAFs could be implicated in the anti-nociceptive action of 5-HT_{1B}Rs.

The role of **5-HT_{1D}** and **5-HT_{1F}** receptors in modulating pain in the spinal cord is still not clearly understood. In the trigeminal DH, sumatriptan and other triptans are effective in the treatment of migraine pain by acting on 5-$HT_{1B,\ D\ and\ F}$ receptors [26]. The anti-migraine effects of triptans are partially mediated by inhibition of nociceptive transmission through several mechanisms, such as decrease of glutamate release from primary afferents [66] and depression of post-synaptic responses to glutamate [67]. Expression of 5-HT_{1D} Rs has been observed in lumbar DRGs, particularly on peptidergic neurons, and in spinal cord DH, suggesting a modulatory action of these receptors also on spinal pain [68]. Immunoreactivity to 5-HT_{1D}Rs undergoes complex changes in adult rat spinal cord DH after induction of peripheral inflammation by Complete Freund's Adjuvant (CFA) injection and after sciatic nerve transection [69]. A recent study has reported that 5-HT_{1D}Rs are involved in the gating of C fiber activity in rat spinal cord and exert a potent inhibition of C fiber-mediated monosynaptic stretch reflex [70].

Similarly to 5-HT_{1A}, also for **5-$HT_{2A/C}$** receptors, both anti- and pro-nociceptive effects have been reported in pain behavioral tests, performed on adult rodents. Intrathecal administration of the 5-HT_2 agonists DOI or alpha-methyl-5-HT decreases the number of flinches in both phases of the formalin test in rats [71, 72] suggesting an anti-nociceptive effect. Anti-hyperalgesic and anti-allodynic actions of 5-HT_2Rs have been described both in carrageenan-induced inflammatory pain [73] and in neuropathic pain models (spinal nerve ligation and chronic constriction injury, CCI), [72, 74, 75]. Conversely, pro-nociceptive effects of 5-$HT_{2A/C}$ receptors have been observed by other studies in the formalin-induced nociception [65], in inflammatory pain produced by CFA [22] and in neuropathy, induced by CCI or administration of nucleoside analogue reverse transcriptase inhibitors [76-78]. Together, these data would suggest an active involvement of 5-$HT_{2A/C}$ receptors in pain central sensitization.

Behavioral studies, performed on adult rodents and investigating the role of **5-HT_3Rs** in pain modulation, have also shown both anti- and pro-nociceptive actions. An anti-nociceptive role has been suggested by earlier studies, reporting that i.t. administered 5-HT_3 agonist 2-methyl-5-HT increases latencies in the tail-flick and hotplate tests [79], while i.t. pretreatment with the 5-HT_3R antagonist ondansetron enhances thermal pain sensitivity in rats [80]. Similarly, antisense oligodeoxynucleotides designed to knock down 5-HT_3Rs decrease 5-HT-induced analgesia on tail-flick reflex [81]. Intrathecal application of 2-methyl-5-HT, a selective 5-HT_3 agonist, inhibits hyperalgesia induced by spinal cord compression [82]. A pro-nociceptive role of spinal 5-HT_3Rs has been evidenced by several studies using behavioral or electrophysiological approaches, especially in conditions of persistent pain. Blocking 5-HT_3Rs with the 5-HT_3R antagonist ondansetron has been proved effective in attenuating formalin-induced behavioral hypersensitivity in rats [83]. Knock-out mice lacking the A subunit of the 5-HT_3R display normal acute pain responses, but attenuated hypersensitivity produced by formalin injection [84]. Intrathecal administration of ondansetron reverses mechanical allodynia in rodent models of central neuropathic pain produced by spinal cord injury [85]. However, the involvement of 5-HT_3Rs in neuropathic pain is still under debate. Spinal administration of the 5-HT_3 antagonist MDL72,222 has no effect on established mechanical allodynia in rats with spinal nerve ligation [86] and knock-out mice for 5-HT_3Rs develop a similar degree of mechanical allodynia as wild type mice, after partial sciatic nerve injury [84]. Accordingly, Peters *et al.* [87] observed that i.t. administration of ondansetron produces no effects on thermal or mechanical hypersensitivity, following spinal nerve ligation in rats. The heterogeneity of the animal models and behavioral tests used in these studies could partially explain the discrepancies observed in the modulatory effects of 5-HT_3Rs. A recent study shows that systemic administration of vortioxetine, a 5-HT reuptake blocker that also antagonizes 5-HT_3Rs, causes robust analgesia in adult CCI mice. Interestingly, the SSRI fluoxetine has no analgesic effects in CCI mice, suggesting that the combination of SSRIs with 5-HT_3 antagonist activity is critical to obtain an analgesic effect in neuropathic pain [88].

Similar differences emerge from the analysis of behavioral studies on the effects of **5-HT_7Rs** in the adult rodent DH, where again both anti- and pro-nociceptive actions have

been reported. The low selectivity of some 5-HT_7 agonists over the 5-HT_{1A} receptor has likely contributed to the heterogeneity of results. Systemic administration of 5-HT_7 agonists (AS-19, MSD-5a and E-55888) in adult rodents produces a dose-dependent anti-nociceptive effect on capsaicin-induced hypersensitivity [89] and mechanical and thermal hypersensitivities evoked by sciatic nerve ligation-induced nerve injury [41, 90]. Similar effects on capsaicin and nerve injury-induced hyperalgesia have been observed after the 5-HT_7 agonist E57431 [91]. Intrathecal administration of the $GABA_A$ receptor antagonist bicuculline reduces the anti-hyperalgesic effects of 5-HT_7R activation, suggesting the involvement of DH GABAergic interneurons [90, 92]. A pro-nociceptive action 5-HT_7Rs has been reported by Rocha-Gonzalez *et al*. [93], showing the increase of flinching during the second phase of the formalin test, induced by low doses of the 5-$HT_{1A/7}$R agonist 5-CT and blocked by the 5-HT_7R antagonist SB-269970. In adult rats submitted to nerve injury, tactile allodynia is reduced by the systemic or spinal administration of SB-269970 [94].

From this overview, it is evident that data obtained from behavioral studies are highly heterogeneous and have not provided, so far, a clear understanding of the role of spinal 5-HTRs in pain modulation. In the following sections, the results derived from electrophysiology studies performed on DRG, superficial and deep DH neurons will be illustrated. Although some discrepancies are still present, the identification of the recorded neurons obtained in some studies has been helpful to clarify some controversial issues.

4. EFFECTS OF SEROTONERGIC RECEPTORS ON DRG NEURONS

4.1. Effects on Isolated DRG Cells

Electrophysiological recordings from rat DRG neurons have confirmed the presence and functionality of most of the 5-HTRs detected with morphological techniques (section 2). Early studies have shown that 5-HT application on DRGs depolarizes most neurons. As reported by Todorovic *et al.* [95], 5-HT depolarizes 82% and hyperpolarizes 4% of the A-type cells, while in C-type cells, 5-HT depolarizes only 41%, but hyperpolarizes 39% of the neurons. Depolarizing responses are mediated by 5-HT_2 or 5-HT_3 receptors, accompanied by an increase or decrease of cell input resistance, respectively. Similarly, 5-HT applied on adult rat cultured DRG neurons evokes a depolarization in about 40% of cells, that is mediated by 5-HT_3Rs [96]. Hyperpolarizing responses, largely confined to Aδ and C-type cells, are due to the activation of 5-HT_1Rs. In acutely isolated rat DRG neurons of the C-type, 5-HT inhibits a high-threshold calcium current and this effect is mediated by 5-HT_{1A}Rs, being mimicked by the application of 8-OH-DPAT [97, 98]. In medium and large-diameter DRG neurons, involved in tactile and proprioceptive sensations, 5-HT potentiates the hyperpolarization-activated cation current (Ih), through the activation of 5-HT_7Rs [99]. This effect increases DRG neuron excitability and possibly neurotransmitter release. In small diameter, capsaicin-sensitive, DRG neurons 5-HT potentiates tetrodotoxin-resistant sodium currents [100]. The effect is mediated by the 5-HT_4 receptor that is positively coupled to adenylyl cyclase and produces an increase in neuronal excitability.

4.2. Primary Afferent Depolarization Mediated by Serotonin

Depolarization of primary afferent terminals (PAD) in DH has been described for both noxious and non-noxious fibers and it is mainly mediated by $GABA_A$ receptors [101-103]. In hemisected spinal cord obtained from neonatal rats, 5-HT produces PAD that is tetrodotoxin-resistant and slower than GABA mediated depolarization [104, 105]. 5-HT induced slow PAD is mimicked by application of the 5-HT_3 agonist m-ChPB, suggesting that these receptors can modulate glutamate release from PAFs through presynaptic terminal depolarization [106]. Furthermore, 5-HT has a depressant action on dorsal root potentials (*i.e*. changes of dorsal root potential that are a reflection of PAD), evoked by stimulation of myelinated cutaneous and muscle afferents. This effect is likely mediated by 5-HTRs different from 5-HT_3 and/or by serotonergic modulation of interposed DH interneurons [105].

5. SEROTONERGIC MODULATION IN SUPERFICIAL DORSAL HORN

5.1. Direct Effects on Neurons

The majority of studies have been performed by recording with the patch-clamp technique from slices of newborn or adult rat spinal cord. Application of 5-HT induces ionic currents in the majority of superficial DH neurons (laminae I-II). In particular, 5-HT produces a slow outward current (that corresponds to neuron hyperpolarization) in the majority of cells and a faster inward current (corresponding to depolarization) in a subpopulation of neurons [107, 108]. Both currents are insensitive to tetrodotoxin, suggesting a direct action of 5-HT on the postsynaptic membrane [108].

The prevalence of the outward or inward current depends on the neuron type, as evidenced by several studies. In young adult rats, the 5-HT induced outward current has been consistently recorded from lamina II excitatory interneurons (vertical and radial cells), from some lamina II inhibitory neurons (islet and tonic central) and, rarely, from lamina I substance P sensitive neurons (including also projection neurons) [109]. The inward current has been found in the inhibitory extended islet neurons, where serotonin mediated excitation is able to evoke action potentials and release of GABA. Similar results have been obtained by Abe *et al*. [108], showing the prevalence of the outward current in vertical cells (excitatory) and of the inward current in islet cells (inhibitory), while small islet cells (including both excitatory and inhibitory interneurons) exhibit both types of currents. Yasaka *et al*. [110] observed the 5-HT induced outward current in most lamina II neurons, with a prevalence in the excitatory vertical and radial cells. Recently, the same type of response has been recorded from excitatory interneurons releasing substance P [111]. In calretinin-eGFP mice, the outward current is present only in excitatory calretinin positive neurons, while no current has been detected in inhibitory calretinin positive cells [112]. All these studies indicate that the 5-HT induced outward current predominates in superficial DH excitatory interneurons, while the inward current could be more frequent in inhibitory neurons. However, experiments performed on adult GAD67-GFP knock-in mice

have found the inward current in a substantial proportion of both GFP positive (inhibitory) and negative (likely excitatory) neurons [113].

The direct effects of serotonin on superficial DH neurons are mediated by different receptors. 5-HT_{1A}Rs are involved in the generation of the outward current, since the application of the 5-HT_{1A} agonist 8-OH-DPAT mimics the response and 5-HT_{1A} antagonists block it [107, 108]. Opening of potassium channels is involved in the generation of this current [108]. The fast inward current is at least partially mediated by the ionotropic 5-HT_3Rs, since it is mimicked by 5-HT_3 agonists mCPBG and 2-Me-5-HT [108, 113].

5.2. Modulation of Synaptic Transmission

The effects of serotonin on excitatory synaptic transmission in superficial DH are biphasic: Hori *et al.* [114] reported that the application of 5-HT on spinal cord slices from neonatal rats decreases the amplitude of the evoked excitatory postsynaptic currents (eEPSCs) and the frequency of spontaneous EPSCs (sEPSCs). These effects are followed, in a subpopulation of neurons, by sustained facilitation of both eEPSCs and sEPSCs. Similar effects have been described also in adult rats, where 5-HT depresses both Aδ and C fiber mediated EPSCs (evoked by dorsal root stimulation), while the frequency of miniature and spontaneous EPSCs shows a depression followed by facilitation [107]. Combining morphological and electrophysiological analysis in young adult rats, Lu and Perl [109] have shown that 5-HT blocks Aδ fiber mediated responses in vertical and radial cells, while C responses are inhibited in most neurons (transient central, islet, extended islet, tonic central, lamina I substance P sensitive or insensitive neurons).

5-HT_1Rs are likely involved in the depression of excitatory transmission induced by 5-HT, while EPSC facilitation could be mediated by 5-HT_2Rs [114]. In neonatal rats, application of the 5-HT_1 agonist 8-OH-DPAT decreases eEPSC amplitude and mEPSC frequency in spinal cord slices [114, 115]. The change of eEPSC paired-pulse and the lack of effect on mEPSC amplitude suggest a presynaptic action of 5-HT_{1A}Rs in this preparation. A 5-HT_{1A}-like receptor could be involved in synaptic modulation in adult rats, since 8-OH-DPAT depresses C-fiber mediated EPSCs, but the effect is insensitive to the 5-HT_{1A} antagonist WAY 100635 [107]. Differently from what observed in trigeminal DH [66], the 5-HT_{1D} agonist PNU109291 and 5-HT_{1F} agonist LY344864 have no effects on evoked EPSCs recorded from superficial spinal cord DH in neonatal rats, while sumatriptan causes a depression of EPSCs that is abolished by WAY 100635 and it is likely mediated by 5-HT_{1A}Rs [115].

The inhibitory synaptic transmission, mediated by GABA and glycine, is potentiated by serotonergic receptors expressed in superficial DH. Application of 5-HT onto spinal cord slices from adult rats enhances GABA and glycine release, as evidenced by the amplitude increase of evoked inhibitory postsynaptic currents (eIPSCs) and the enhancement of spontaneous IPSC amplitude and/or frequency [108, 116]. 5-HT_3 receptors, able to excite GABAergic interneurons (section 5.1), are importantly involved in the modulation of inhibitory transmission. The 5-HT_3 agonist 2-Me-5-HT increases eIPSC amplitude and mIPSC frequency in GAD67-GFP negative neurons from mouse spinal cord, indicating that 5-HT_3Rs facilitate GABA and glycine release onto excitatory interneurons [113]. Similarly, the 5-HT_3 agonist mCPBG enhances sIPSC frequency and amplitude in lamina II neurons from adult rats, consistently with the expression of 5-HT_3Rs at presynaptic terminals and soma-dendritic sites of inhibitory interneurons [108, 116]. 5-HT_2Rs are also involved in 5-HT induced potentiation of inhibitory transmission: activation of 5-HT_2Rs enhances both frequency and amplitude of sIPSCs [116] and directly modulates $GABA_A$ receptors, potentiating the GABA induced current [117].

Interestingly, serotonin reuptake inhibitors can mimic the effects of 5-HT application on excitatory synaptic transmission in DH. Fluvoxamine reduces Aδ and C fiber mediated EPSCs in young adult rats, through a presynaptic mechanism. The depression of A fiber EPSCs is decreased in the presence of 5-$HT1_A$ and 5-HT_3 antagonists, while the effect on C fiber EPSCs is reduced by 5-HT_{1A} antagonists only. sEPSC and mEPSC frequency are increased by fluvoxamine, likely reflecting an increase of glutamate release from interneurons [118].

6. EFFECTS OF SEROTONERGIC RECEPTORS ON DEEP DORSAL HORN NEURONS

6.1. *In Vivo* Experiments

Much of the knowledge about the specific effects of 5-HT in the deep DH (laminae III-VI) has been obtained from *in vivo* electrophysiological experiments on adult animals, with extracellular recordings and iontophoretic application of serotonergic drugs. In general, these studies indicate that 5-HT depresses nociceptive responses of deep DH neurons in primates [119], cats [120] and rats [121, 122]. The recorded cells are mostly wide dynamic range neurons (WDR), receiving both non-noxious and noxious inputs. In some cases, a potentiating effect of 5-HT on nociceptive responses has been observed [121, 123, 124].

5-HT_{1A}, 5-HT_{1B} and 5-HT_3 receptors have been involved in 5-HT induced depression of WDR responses in rat DH [125, 126]. In particular, 5-HT_{1A} agonist 8-OH-DPAT inhibits the responses evoked by skin electrical stimulation and mediated by both Aδ and C fibers, while the 5-HT_{1B} agonist CP93,129 depresses Aδ responses and increases post-discharge activity [127, 128]. Similarly, the 5-HT_{1A} agonist F 13640 depresses WDR responses evoked by pinch stimuli [129]. 5-HT_3Rs exert inhibitory effects on WDR, that could be indirectly due to the excitation of GABAergic neurons, since they are blocked by the $GABA_A$ antagonist bicuculline [125]. Recently, the facilitation of WDR neuron responses by 5-HT_3Rs has been described during spinal cord development [130]. While tactile activity in WDR neurons is facilitated through life by serotonergic fibers, nociceptive processing is potentiated in younger rats and inhibited in the adults. Facilitation of tactile processing in both young and adult rats and of nociception in younger animals is critically mediated by 5-HT_3Rs.

5-$HT_{2A/C}$Rs could play a pro-nociceptive role in deep DH, since the 5-HT_{2A} antagonist ketanserin and the 5-$HT_{2A/C}$ antagonist ritanserin decrease rat WDR neuron responses to

electrical, mechanical and thermal stimulation, while 5-$HT_{2A/C}$ agonist DOI potentiates these responses [131]. Conflicting results have been obtained by Liu *et al*. [132], showing that ketanserin and RS 102221, antagonists of the 5-HT_{2A} and 5-HT_{2C} receptors, respectively, are effective in blocking the depressing action of 5-HT on C fiber mediated responses of rat WDR neurons. These data would indicate an anti-nociceptive effect of 5-HT_2 receptors in deep DH.

6.2. Effects on Deep Dorsal Horn Neurons *in vitro*

Exogenous 5-HT generally depresses excitatory postsynaptic potentials (EPSPs), evoked by nociceptive PAF stimulation and recorded from deep DH neurons in neonatal rats, in the hemisected spinal cord or in slices [133, 134]. EPSP facilitation has been observed in a few DDH neurons [134]. The depressing effect of 5-HT is mimicked by the 5-HT_1 agonists 8-OH-DPAT and 5-CT. Application of 8-OH-DPAT, able to activate also 5-HT_7Rs, induces EPSP facilitation in a subpopulation of deep DH neurons, that is blocked by the 5-HT_7 antagonist clozapine [135]. The same study reports the inhibiting effect exerted on EPSPs by 5-HT_{1B}Rs, while 5-$HT_{2A/C}$ and 5-HT_3 agonists have modest facilitatory effects. In absence of any agonist, the 5-HT_{1A} antagonist NAN-190 often facilitates synaptic responses, suggesting a tonic activation of 5-HT_{1A}Rs by endogenous serotonin. In neonatal rat hemisected spinal cord, Worsley *et al*. [136] observed a preferential inhibitory effect of 5-HT on prolonged polysynaptic EPSPs recorded from deep DH neurons, some of which express NK1 receptors. The depression of the slow EPSPs could be partly due to the serotonergic modulation of NMDA receptors, as reported by other studies [137, 138].

Shay and Hochman [139] described an interesting mechanism by which 5-HT alters the multi-segmental convergence patterns in deep DH dorsal horn. In the isolated spinal cord from neonatal rats, 5-HT depresses inhibitory postsynaptic potentials (IPSPs), evoked by stimulating adjacent dorsal roots, in neurons receiving predominant inhibitory inputs, while the excitatory responses from the homologous root are unaffected. In neurons receiving predominant excitation, 5-HT decreases the EPSPs evoked by stimulation of roots from all segments. These results suggest that 5-HT may induce anti-nociception also by reducing the inhibitory inputs surrounding the central excitatory zone, decreasing the sensory contrast of the noxious stimulus.

7. ROLES OF SEROTONERGIC RECEPTORS IN SYNAPTIC PLASTICITY

Several studies have reported the active role of 5-HTRs in mediating synaptic plasticity in spinal cord DH. Some of these findings will be summarized below.

The 5-HT_{1A} agonist F13640 depresses the progressive increase of action potential firing in WDR neurons during repetitive activation of C fibers (a phenomenon termed "wind-up") [129]. Modulation of NMDA receptors by 5-

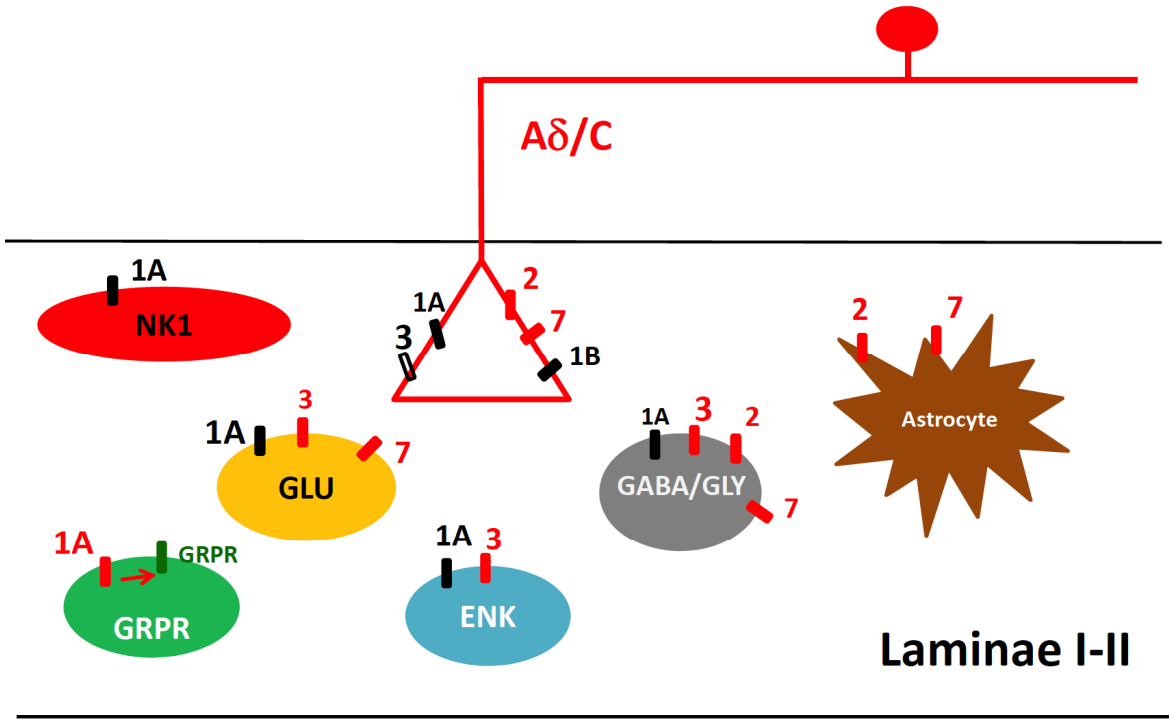

Fig. (1). Schematic representation of 5-HTR expression and function in superficial dorsal horn. Receptors with inhibitory actions are represented in black, receptors with facilitating effects are shown in red. 5-HT_3Rs on primary afferent terminals could exert both effects. NK1: lamina I neurons expressing NK1 receptors, including projection neurons; GLU: excitatory, glutamatergic interneurons; GRPR: excitatory interneurons involved in itch transmission and expressing the receptor for the GRP peptide; ENK: enkephalinergic neurons; GABA/Gly: inhibitory interneurons releasing GABA and/ or glycine. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

HT_{1A}Rs could be a mechanism by which these receptors control the cumulative depolarization that is associated with a central temporal summation. Long-term synaptic modifications, such as LTP or LTD have been described in DH [140, 141] and the serotonergic descending system has been involved in both the induction and the direction of synaptic plasticity [142]. In neonatal rats, conditioning stimulation of primary afferents induces LTD in most recorded deep DH neurons and LTP in only a minority. In the presence of 5-HT or the 5-$HT_{1A/1B}$ agonist 5-CT, the incidence of LTD is significantly increased [140]. The inhibition of postsynaptic calcium channels by 5-$HT_{1A/B}$ receptors could contribute to altering the direction of synaptic plasticity.

In the superficial dorsal horn of postnatal rats, Li and Zhuo [143] have shown that high doses of 5-HT inhibit evoked EPSCs, while low doses or a selective 5-HT_2 agonist induce EPSC facilitation, persisting after washout. The 5-HT induced potentiation of EPSCs is obtained by postsynaptic recruitment of AMPA receptors through cAMP signaling, by adding new receptors to both silent and AMPA receptor-mediated synapses [144].

Finally, an interesting role has been proposed for dorsal horn 5-HT_{1A}Rs in the modulation of itch transmission [145]. Opposed to the prevalent inhibitory role played in the nociceptive processing, 5-HT_{1A}Rs exert a facilitatory action on itch. 5-HT_{1A}Rs are present on GRPR neurons, excitatory interneurons expressing the receptor for the GRP peptide, which have been indicated as key elements for itch transmission in the spinal cord. Binding of 5-HT to 5-HT_{1A}Rs induces crosstalk between these receptors and GRP receptors, potentiating their response to GRP and increasing neuron excitability. This, in turn, would facilitate the transmission of itch information to projection neurons and higher centers.

CONCLUSION

A summary of the possible locations and functions of the main 5-HTRs expressed in superficial DH is represented in Fig. **1**. Nociceptive fibers of the Aδ and C-type express several 5-HTRs on their presynaptic terminals in the DH. The expression pattern may vary, depending on the fiber type. In general, activation of presynaptic 5-HT_{1A} and 5-HT_{1B} produces a decrease of glutamate release, while 5-HT_2 and 5-HT_7 could exert a facilitating role (to be confirmed). The ionotropic 5-HT_3Rs are involved in primary afferent depolarization and their effect on glutamate release could be variable [101, 102].

Postsynaptic 5-HTRs expressed on superficial DH neurons likely include all types described in this review. 5-HT_{1A}Rs may be prevalently expressed on excitatory neurons (including also NK1 expressing projection neurons), where they cause neuron hyperpolarization. 5-HT_3Rs could exert a depolarizing action prevalently on inhibitory interneurons, leading to an increase of GABA and glycine release. In GRPR neurons, involved in itch transmission, 5-HT_{1A}Rs potentiate the response to GRP and increase neuron excitability. Astrocytes express some 5-HTR types, such as 5-HT_2 and 5-HT_7, but their functions are still not known.

From this summary, it is evident that the characterization of the role of the different 5-HTRs in the modulation of DH nociceptive circuits requires further studies. It is still not clear, for example, what is the action of 5-HTRs on different DH neuron populations, and in particular on projection neurons, in conditions of acute and chronic pain. The effects of endogenously released 5-HT on the different neuronal populations need also to be investigated. In the next future, the use of transgenic animals with fluorescently labelled neurons, the selective neural stimulation through opto- or chemogenetics and the availability of more selective pharmacological tools will be critical to gain a better understanding of these topics.

LIST OF ABBREVIATIONS

Chemical Names of the Compounds Cited in the Text

AS-19	=	(2*S*)-(+)-5-(1,3,5-Trimethylpyrazol-4-yl)-2-(dimethylamino)tetralin
CP 93129	=	1,4-Dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5*H*-pyrrol[3,2-*b*]pyridin-5-one dihydrochloride
5-CT	=	5-Carboxamidotryptamine
DOI	=	2,5-Dimethoxy-4-iodoamphetamine
E-55888	=	N,N-Dimethyl-2-[3-(1,3,5-trimethylpyrazol-4-yl)phenyl]ethanamine
E57431	=	(2-(2-(dimethylamino)ethyl)-4-(1,3,5-trimethyl-1H-pyrazol-4-yl)phenol hydrochloride)
F13640	=	3-chloro-4-fluoro-phenyl)-[4-fluoro-4-{[(5-methyl-pyperidin-2-ylmethyl)-amino]-methyl}piperidin-1-yl]-methanone
[125I]GTI	=	serotonin-O-carboxy-methyl-glycyl-[125I]tyrosinamide
Ketanserin	=	3-[2-[4-(4-Fluorobenzoyl)-1-piperidinyl]ethyl]-2,4[1*H*,3*H*]-quinazolinedione tartrate
LY344864	=	*N*-[(3*R*)-3-(Dimethylamino)-2,3,4,9-tetrahydro-1*H*-carbazol-6-yl]-4-fluorobenzamide hydrochloride
mCPBG	=	1-(m-Chlorophenyl)-biguanide
MDL 72222	=	lαH,3α,5α-H-tropan-3-yl 3,5-dichlorobenzoate
MSD-5a	=	(dimethyl-[2-(6-phenyl-pyridin-2-ylsulfanyl)-ethyl]-amine hydrochloride
NAN-190	=	1-(2-Methoxyphenyl)-4-(4-phthalimidobutyl)piperazine
8-OH-DPAT	=	8-hydroxy-2-(di-n-propylamino) tetralin
PNU109291	=	(*S*)-3,4-Dihydro-1-[2-[4-(4-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-methyl-1*H*-2-benzopyran-6-carboxamide
Ritanserin	=	6-[2-[4-(bis(4-Fluorophenyl)methylene]-1-piperidinyl]ethyl]-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one

RS102221 = 8-[5-(2,4-Dimethoxy-5-(4-trifluoromethylphenylsulphonamido)phenyl-5-oxopentyl]-1,3,8-triazaspiro[4.5]decane-2,4-dione

RU 24969 = 5-Methoxy-3-(1,2,5,6-tetrahydro-4-pyridinyl)-1H-indole.

SB-269970 = (*R*)-3-[2-[2-(4-Methylpiperidin-1-yl)ethyl] pyrrolidine-1-sulfonyl]phenol

WAY-100635 = *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl}-*N*-(2-pyridinyl) cyclohexanecarboxamide

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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