SEROTONIN 5-HT₃ RECEPTOR MEDIATION OF PAIN AND ANTI-NOCICEPTION: IMPLICATIONS FOR CLINICAL THERAPEUTICS

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The involvement of the serotonergic system in pain and anti-nociception has long been recognized. Throughout the nervous system, serotonin (5-HT) exerts effects through heterogeneous populations of receptors that have recently been categorized into distinct "families" based upon their molecular properties. Of these, the 5-HT3 receptor is distinct in that it is inotropic, mediating a sodium current, and thus is exclusively excitatory, irrespective of the tissue(s) in which it is localized. Widely distributed within the brain, spinal cord, peripheral neurons and extraneural tissues, 5-HT3 receptors have been localized to several anatomical loci within the peripheral and central neuraxes that subtend the afferent transmission and efferent modulation of pain. This review provides an overview of the anatomy and physiology of 5-HT3 receptors and focuses upon the work of numerous groups, as well as summarizing our previous and ongoing studies, that have investigated the role of 5-HT3 receptors in pain and anti-nociception. Data from in vitro studies of pharmacologic function and molecular mechanisms, ex vivo bioassays and animal studies are addressed. Of particular interest are those findings relevant to the possible utility of 5-HT3-acting agents in treating human pain and the results of clinical trials employing 5-HT3 selective drugs for applied therapeutics.

Keywords: Neurotransmitter, Serotonin, 5-HT3, spinal cord, pain.

A copious neurotransmitter and neuromodulator, serotonin (5-hydroxytryptamine, 5-HT) is found in the central nervous system, peripheral nervous system, platelets and enteric neural tissues (1). The involvement of the serotonergic system in the processing of pain has long been recognized. In general, the central 5-HT neuraxis subtends analgesia through bulbospinal and supratentorial pathways. In contrast, peripheral serotonergic activity is strongly pro-algesic. The actions of 5-HT are mediated through no less than twelve distinct receptor types that are heterogeneously distributed throughout tissues in which 5-HT exerts effect, although not all appear to be present in humans (2) (Table 1).

Initially, 5-HT receptors were characterized according to pharmacologic profiles of ligand binding, agonist and antagonist affinity and effect(s) (3). However, recent advances in structural molecular biology and cell physiology have revised the classification scheme based upon structural homology and transcriptional code and have demonstrated the existence of distinct "families" of 5-HT receptors (4, 5) as illustrated by Table 2.

The 5-HT₃ Receptor

Of these receptors, only the 5-HT₃ receptor is not metabotropically linked to a G-protein-mediated system to catalyze neuronal activity through a second-messenger-based cascade (6). Rather, it is a ligand-gated ion channel, existing in two probable subtypes, as a structural pentamer that surrounds a central sodium (Na^+) ionophore (7). Serotonin 5-HT₃ receptors have been peripherally localized to the pre- and post-ganglionic autonomic fibers, nodose ganglion, vagal afferents, within the dorsal root ganglion and terminals of C-fiber and non-C fiber nociceptive afferents (8, 9). Centrally they are found in the superficial laminae of the spinal dorsal horn and several supraspinal loci including the brainstem area postrema, solitary tract nuclei, the nucleus ambiguus and in the brain at the nucleus accumbens, amygdala, habenula, hippocampus and diffusely throughout the cortex (10, 11). Two subunits of receptor 5HT₃ have been described with 5HT₃A found on both central and peripheral nervous system neurons while 5HT₃B is limited to peripheral nervous system neurons (12). Current data indicate that the 5-HT₃B subunit functions only in conjunction with 5-HT₃A and not as a homomeric receptor (13).

Pharmacology

Serotonin is the endogenous ligand for 5-HT₃ receptors. The principal agonists acting at both central and peripheral 5-HT₃ sites are 2- methyl-serotonin (2-methyl-5-HT) and 2-chlorophenyl-biguanide (14,15). In contrast, the agonist trimethylserotonin, a quaternary 5-HT analog, is impenetrable to the blood-brain barrier, and is therefore pharmacokinetically selective for peripheral 5-HT₃ sites (16). These agonists have not been clinically employed, and thus their utility is limited to experimental investigations of 5-HT₃-mediated effects.

Initially, zacopride was developed as an antagonist with selective affinity at 5-HT₃ sites. Subsequently, other agents, namely granisetron, ondansetron and tropisetron were developed and shown to exert more potent antagonist activity at 5-HT₃ receptors *in vitro* and *in vivo* (although tropisetron acts as an antagonist at 5-HT₄ receptors, as well). Serotonin 5-HT₃ receptor antagonists freely penetrate the blood-brain barrier and block central 5-HT₃ receptors: 1) in the area postrema reducing the emetic effects induced by radio- and chemo-therapeutics (17) and, 2) within the hippocampus and limbic fore-

brain where 5-HT$_{3A}$ specific antagonists have been shown to reduce anxiety and may affect GABAergic function in synaptic plasticity, learning and memory (18).

Within the dorsal root ganglion and peripherally, 5-HT$_3$ receptor antagonists act at 5-HT$_3$ sites on inflammation associated C- and non-C-fibers to produce anti-nociceptive effects by diminishing the 5-HT-induced release of substance P (and/or other mediators of neurogenic inflammation) and perhaps by altering the expression or sensitivity of post-synaptic neurokinin receptors within the nociceptive neuraxis (20). The action of 5-HT$_3$ receptor antagonists at serotonergic enteric neurons has been shown to reduce secretion and diarrhea caused by increased intestinal serotonin content (21) (Table 3).

Irrespective of tissue, the 5-HT$_3$ receptor mediates a depolarizing sodium current, leading to Ca^{++} influx, a rise in cytosolic Ca^{++} and ultimately neuronal excitation (7, 22). Similar to other inotropic receptors, the post-synaptic response is fast, but unlike typical inotropic receptor-mediated responses, the excitatory effects mediated by the 5-HT$_3$ receptor are significantly slower (22, 23). As depicted by Fig.1, 5-HT$_3$ receptor activation also activates a calcium-calmodulin mediated activation of protein kinase(s) that may subserve nuclear effects (e.g., proto-oncogene induction) and induces an increase in nitric oxide (NO) that may underlie a variety of intracellular (e.g., altered membrane physiochemistry, possible modification of NK-1 receptors, engagement of cGMP-mediated mechanisms) and extracellular effects (e.g., vasodilation, post-synaptic neurotransmission) (24). The receptor is allosterically modulated by local anesthetics and gonadal steroids (25, 26) and is capable of pharmacologic alteration in sensitivity, most probably through change in one or more of the amino acid residues in one of the transmembrane segments that surround the central ion pore (27, 28).

Our initial interest in the possible role of the 5-HT$_3$ receptor in pain physiology began some 15 years ago when it was revealed that administration of prototypic 5-HT$_3$ receptor antagonists produced distinct patterns of analgesia in various nociceptive assays in rodents. Systemic administration of these drugs produced moderate anti-nociceptive effects against chemical-inflammatory and thermal pain, but not against mechanical pressure or distention (29). This was consistent, in part, with the previously demonstrated relative stimulus specificity of the serotonergic system against these types of pain (30, 31). However, it was unclear whether these effects were mediated by a

Table 1. *Serotonin receptors: Anatomic distribution*

5-HT Receptors		Location
5-HT1	A	Hippocampus, septum, amygdala, raphe nuclei, neocortex, hypothalamus, substantia gelatinosa
	B	Substantia nigra, basal ganglia, subiculum, suprachiasmatic nucleus
	D	Substantia nigra, basal ganglia, superior colliculus, raphe nuclei, cerebellum, entorhinal cortex
	E	
	F	
5-HT2	A	Claustrum, olfactory tubercle, cortex, amygdala, hippocampus, hypothalamus
	B	
	C	Choroid plexus, globus pallidus, substantia nigra
5-HT3	subunit A	Area postrema, spinal cord, limbic system, solitary tract
	subunit B	Dorsal vagal nerve, trigeminal nerve, postganglionic autonomic neurons, G.I. tract neurons.
5-HT4		Colliculus, hippocampus
5-HT5	A	Cortex, hippocampus, habenula, olfactory bulb, cerebellar granular layer
	B	
5-HT6		Limbic system, pial veins
5-HT7		Limbic system, thoracic & upper lumbar spinal cord, hypothalamus, jejunum ileum, pial veins, veins of smooth muscle

Table 2. *Molecular "familes" of 5-HT3 receptor*

Family	Receptor(s)	Action
Gαi/o G protein	5-HT1A,B,D ,E & F & 5-HT5A,B	Inhibit adenylyl cyclase
Gαq/11 G protein	5-HT2A, B, & C	Activate phospholipase C
Ion channel	5-HT3 subunits A & B	Ligand gated ion channel
Gs G protein	5-HT4, 5-HT6 & 5-HT7	Activate adenylyl cyclase

Table 3. *5-HT3 receptor Pharmacology*

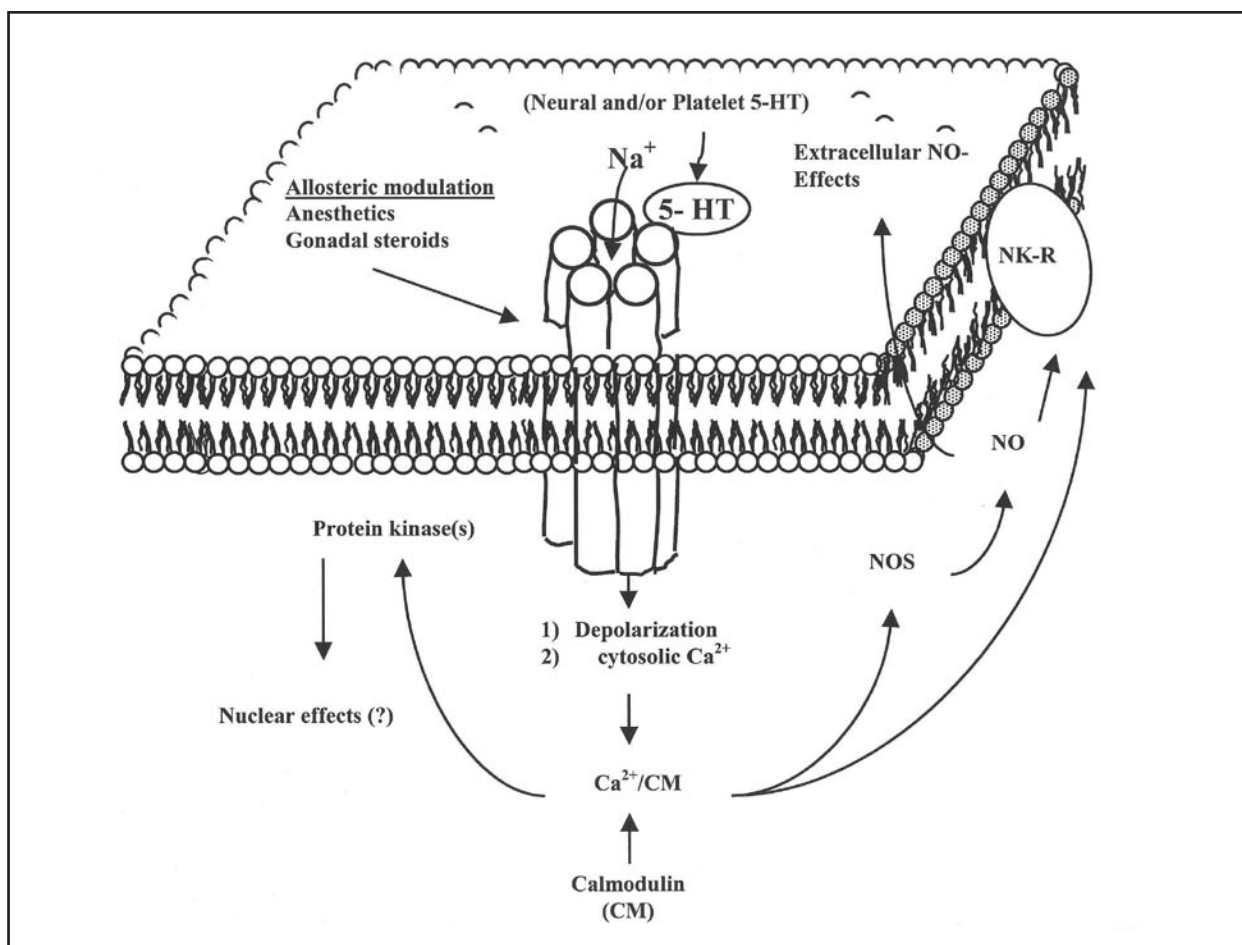| 5-HT3 Receptor Agonist	5-HT3 Receptor Antagonist
5-carboxyamidotryptamine	Dolasetron
Chlorobiphenylbiguanide	Ondansetron
5-HTT(Q)	Granisetron
2-methyl-5-HT	Tropisetron
Neurokinin	R-Zacopride (also 5-HT4)
Phenylalanine 107	Gonadal Steroids
YM-31636	Mitirzapine
PSAB-OFP	Clozapine
Quipazine	Anpiroline (also 5-HT1B)
SR57227A	Y-25130
	ADR-851S
	ADR-851R

Fig 1. *Diagrammatic representation of 5-HT3 receptor-mediated events. The 5-HT3 receptor exists as a poly-loop, transmembrane pentamer surrounding a central sodium (Na+) ion pore. Activation of the 5-HT3 receptor by neural, platelet and/or blood-borne 5-HT induces an inward Na+ current that leads to neuronal depolarization and a rise in free cytosolic calcium (Ca2+). Calcium couples with calmodulin to cleave membrane-bound nitric oxide synthase (NOS) into the cytoplasm to catalyze the formation of nitric oxide (NO). Nitric oxide can diffuse extracellularly to facilitate local neurotransmission and exert physiologic actions upon non-neural tissues (e.g., vasodilation). As well, NO and the calcium-calmodulin complex may activate spare fractions of sequestered neurokinin NK-1 receptors. The activated calcium-calmodulin complex can also activate Ca2+-dependent protein kinase(s) to initiate subsequent intracellular reactions and perhaps induce effects within the nucleus (e.g., activation of specific proto-oncogenes, including c-fos). The 5-HT3 receptor is allosterically modifiable by local anesthetics and gonadal steroids, and may require sensitization by exposure to the endogenous ligand, 5-HT, to facilitate subsequent sensitivity to other agonist compounds.*

central mechanism, peripheral action, or both. The intracerebroventricular and intrathecal delivery of these agents were notably ineffective in eliciting any anti-nociceptive response, whatsoever. In fact, intrathecal delivery produced a significant reduction in the nociceptive threshold to both chemical-inflammatory, and to a lesser extent, thermal pain (29). These findings strongly suggested that central 5-HT_3 receptors were in some way involved

with analgesic mechanisms. The anatomic localization of these receptors within the superficial laminae and substantia gelatinosa of the spinal cord and on nocisponsive C-fiber and non-C-fiber primary afferents in the periphery certainly support such conclusions (8, 9).

Thus, the observed analgesic effects of systemically administered 5-HT_3 antagonist drugs were likely due to the blockade of some peripheral, rather than

central 5-HT_3 receptor-mediated mechanism, that was a component of the nociceptive response.

Peripheral 5-HT_3 Receptors

Further definition of the role of peripheral 5-HT_3 receptors was provided by studies that demonstrated that peripheral microinjection of prototypic 5-HT_3 antagonists produced dose-dependent anti-nociceptive effects against chemo-

inflammatory pain produced by intra-plantar formalin, acetic acid or inoculation with Freund's adjuvant (*mycobacterium butyricum)* (32). As release of peripheral 5-HT is responsible, at least in part, for the effects of these substances, it became increasingly apparent that 5-HT$_3$ receptors were important in mediating components of this response. Subsequent work by Sufka et al (33) revealed that direct delivery of 5-HT into peripheral tissue evoked concentration-dependent patterns of inflammation and pain. However, different aspects of the inflammatory response (e.g., pain, flare, wheal) were not uniformly subserved by a single population of 5-HT receptor. Consistent with these findings, studies have confirmed that 5-HT$_2$ and perhaps 5-HT$_1$ and 5-HT$_4$ (34) receptors mediate a considerable extent of the vascular response (edema, flare, wheal) while 5-HT$_3$ receptors appeared to be responsible for the nociceptive effects and perhaps also the maintenance of a latent, secondary phase of inflammation (9, 32, 33).

In this model, insult to peripheral tissue produces membrane disruption to induce the production and release of a variety of substances that are pro-inflammatory. These include arachidonic acid and resultant intermediate- and end-products of the cyclo-oxygenase-catalyzed cascade (e.g., prostaglandins), bradykinin, increased concentration of H$^+$ ion, and the cytokines (interleukins IL-1, 2, 6 and tumor necrotic factor, TNF). As well, membrane disruption yields increased concentrations of nitric oxide (iNO). These substances act as potent vasodilators. Derangement of the membrane structure of the micro- (and macro) vascular bed further facilitates extravasate effects. This vascular disruption, together with direct activating effects of the cytokines, causes platelet de-granulation, resulting in the release of 5-HT. Additionally, free, blood-borne 5-HT extravasates into the peripheral tissue bed (35). The increased local concentration of 5-HT is within the physiologic range to activate 5-HT$_3$ receptors located on the terminals of the free endings of nocisponsive afferents, depolarizing these fibers and initiating a nociceptive response. It is of interest that typical 5-HT$_3$-mediated algesic responses are not elicited by peripheral or visceral administration of the prototypic agonist 2-methylserotonin (27, 36). It may be that

peripheral 5-HT$_3$ receptors require direct serotonergic stimulation for activation. It is also possible that 5-HT-induced activation of peripheral 5-HT$_3$ receptors initiates a catalytic cascade that either "unmasks" sequestered populations of 5-HT$_3$ sites from a non-membrane pool and/or instigates transcriptional or translational process that code for the *de-novo* synthesis of additional 5-HT$_3$ receptors. Following such un-masking or synthesis, it appears that these receptors are sensitive to other agonists (27).

Persistent activation of 5-HT$_3$ receptors on peripheral and visceral afferents also appears to subserve a component of allodynia, apparently via interaction with 5-HT$_4$ receptor-mediated mechanisms (37). While the activation of non-C-fiber afferents does not appear to contribute to a serotonergically mediated inflammatory response (9), the prolonged activation of C-fibers may result in the retrograde release of Substance-P and glutamate into the peripherally innervated tissue. The 5-HT$_3$ receptor also can mediate the production of NO, an effect elicited "downstream" following the subsequent activation of a number of reactions that ultimately leads to an increased expression of the catalyzing enzyme, nitric oxide synthase (NOS) (Fig. 1) (38). Together, peripherally released Substance-P and elevated levels of NO exert strongly vasodilatory effects, thereby perpetuating inflammation. Thus, after the initial, acute inflammatory response, it appears that a secondary, neurogenic inflammation occurs that is partially mediated by 5-HT$_3$ receptor-related mechanisms (24, 32- 34).

Central 5-HT$_3$ Receptors

While peripheral 5-HT$_3$ receptors subserve mechanisms of nociception and perhaps contribute to secondary inflammation, central 5-HT$_3$ receptors play a different role. The central 5-HT system is a critical substrate in analgesia. As schematically depicted in Fig. 2, serotonergic fibers from the raphe nuclei of the rostro-ventral medulla project via the dorsolateral funiculi to innervate targets throughout the superficial dorsal horn of the spinal cord (39). Activation of these descending 5-HT pathways directly or through centrifugal opioid mechanisms originating from the midbrain periaqueductal gray (PAG) causes the release of 5-HT at synaptic connections upon both nociceptive

afferents and pools of local and segmental interneurons within the cord to produce analgesia (30, 31). Serotonin 5-HT$_3$ receptors have been identified in the superficial laminae of the spinal dorsal horn (11, 12). Intrathecal administration of 5-HT$_3$ receptor antagonists blocked 5-HT-induced analgesia and produced a moderate hyperalgesic response (29). As well, the reduction of neurotrophin-induced, descending anti-nociception by administration of 5-HT$_3$ receptor antagonists suggested that 5-HT$_3$ receptors are involved with the expression of centrifugal, serotonergic pain modulation at the spinal level (40). Intraspinal administration of the 5-HT$_3$ receptor agonist, 2-methylserotonin produced significant dose-dependent analgesia against chemical/inflammatory and thermal pain, but not mechanical pain. The specificity of this effect was demonstrated in that it was blocked by pre-treatment with 5-HT$_3$ receptor antagonists. However, the effect was also reduced, to varying extent, by administration of the GABA receptor antagonist bicuculline and the opioid antagonist naloxone. This suggested that the analgesic effects produced by activation of 5-HT$_3$ receptors are reliant upon spinal GABAergic and opioid mechanisms (41).

Based upon the known pharmacology and anatomy of the dorsal horn, it is likely that descending raphe-spinal 5-HT pathways release 5-HT that binds to 5-HT$_3$ receptors on interneurons within the superficial laminae (42). Activation of 5-HT$_3$ receptors depolarizes these neurons causing the release of GABA and opioids (e.g., enkephalin, dynorphin) to inhibit primary and/or second-order nocisponsive neurons (41). The relative stimulus-specificity of this analgesia against inflammatory, and to a lesser degree thermal and mechanical pain, may represent the selectivity of the 5-HT system as a "labeled line" for particular nociceptive modulation or may reflect the sensitization of peripheral afferent fibers to chemical changes in innervated tissue as a consequence of noxious thermal and/or mechanical stimuli (30, 31, 43). It is tempting to hypothesize that the 5-HT$_3$-receptor system plays a unique role in both the afferent processing of chemical/inflammatory pain and the efferent modulation of this type of nociception by the central serotonergic system. However, such possibility remains purely speculative, at best.
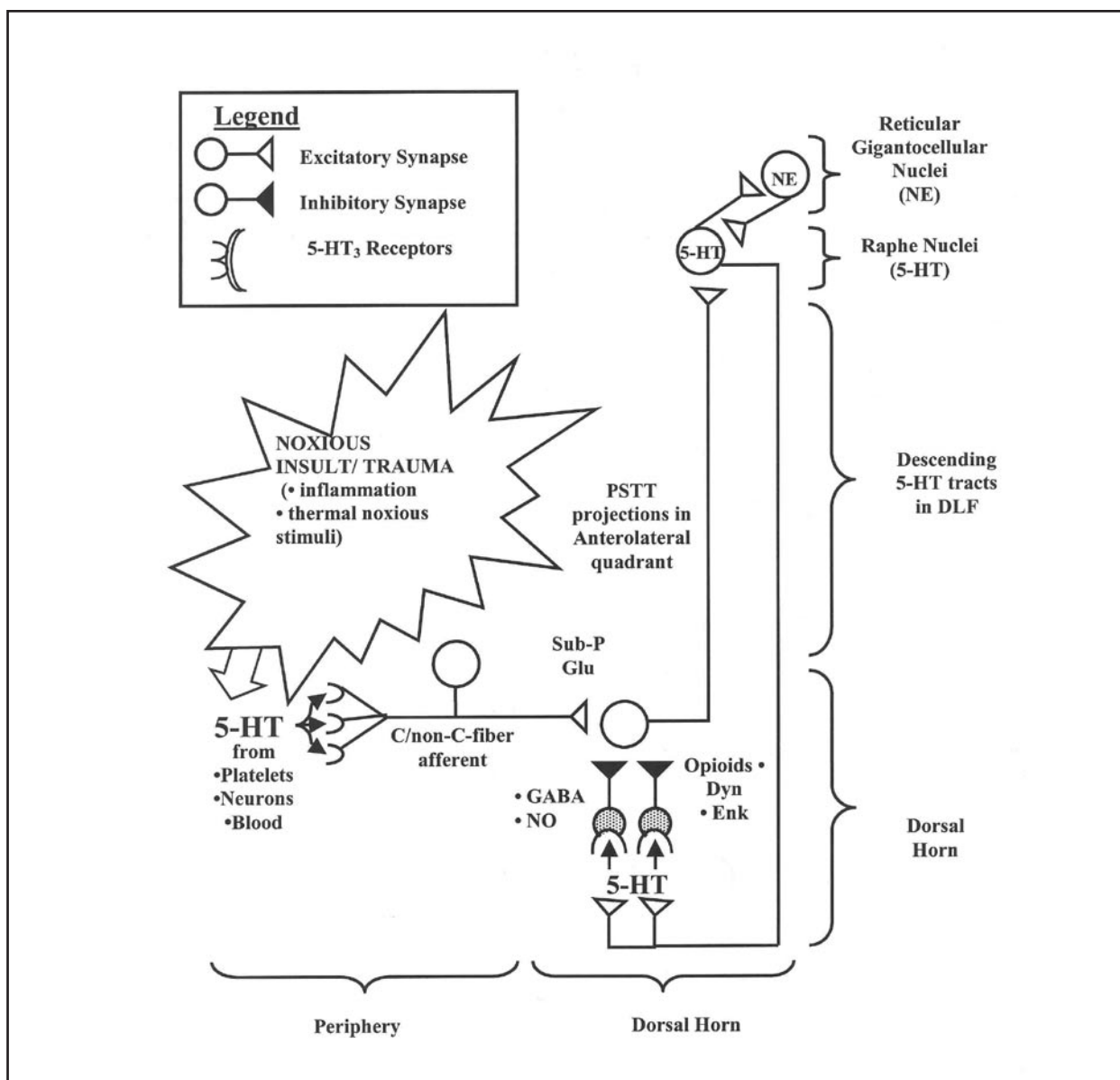
Fig 2. *Schematic of the putative neuraxis involved in 5-HT3 receptor-mediated nociception and pain modulation. In this model, inflammatory (and perhaps thermal) noxious stimuli evoke a local release of serotonin (5-HT) from peripheral stores (e.g., platelets, neurons, extravasation from blood) to activate 5-HT3 receptors on free endings of peripheral C- and non-C-fiber nociceptive afferents. Depolarization of these fibers causes a release of Substance-P, glutamate and other pro-nocisponsive substances both within the superficial dorsal horn, and perhaps retrogradely into the innervated tissue(s). Activation of second-order afferents of the paleospinothalamic tract (PSTT) engage serotonergic neurons of the brainstem Raphe nuclei. Interaction occurs between Raphe 5-HT and noradrenergic neurons of the reticular gigantocellular nuclei; as well, these brainstem loci can be engaged by centrifugal mechanisms emanating from the richly opioid periaqueductal grey area of the midbrain. Descending 5-HT tracts terminate in the superficial laminae of the spinal dorsal horn. Spinally-released 5-HT binds to 5-HT3 receptors on populations of inhibitory interneurons, evoking the release of GABA, NO and opioids that modulate excitatory transmission in nociceptive afferents. (Details in text)*
Note that 5-HT can also act directly to inhibit primary and second-order nociceptive afferents, although these effects do not appear to be mediated by 5-HT3 receptor-specific mechanisms
(Abbreviations: DLF: dorsolateral funiculus; Dyn: dynorphin; Enk: enkephalin; GABA: gamma amino butyric acid; NE: norepinephrine; NO: nitric oxide; PSTT: paleospinothalamic tract)

Implications for Clinical Practice

While animal data reveal a role for peripheral and central 5-HT$_3$ receptors in pain and pain modulation, the direct translation of such studies into paradigms that are appropriate for clinical therapeutics against pain warrant caution. It is well known that serotonergic agents (e.g., selective re-uptake blockers, autoreceptor agonists) can be effectively used as both adjunctive and primary analgesics (44). However, the protocols employed in animal studies that elicited effective 5-HT$_3$ receptor-mediated analgesia utilized microinjection of antagonists into the periphery or central delivery of agonists to the spinal cord. Such procedures are not wholly viable as techniques for human trials, and even if conducted, do not represent facile approaches to pain control. Yet, animal studies have provided considerable insight to the possibility that the 5-HT$_3$ receptor system could be targeted for clinical pain therapeutics.

A number of studies have examined the potential clinical effects and utility of drugs that act at central and/or peripheral 5-HT$_3$ receptors. While spinal 5-HT$_3$ receptors appear to play a role in mediating the bulbospinal analgesia of 5-HT and the descending effects of peripherally and centrally administered opiates, the specific targeting of central 5-HT$_3$ sites through systemic administration of agents appears to be particularly difficult from a clinical standpoint. As previously discussed, to date, there is little evidence of the use of specific 5-HT$_3$ receptor agonists (e.g., 2-methyl-5-HT) in clinical application. And, while the use of non-receptor-selective serotonergic compounds (e.g., tryptophan, 5-HT re-uptake inhibitors) may engage 5-HT$_3$ mediated mechanisms, such a "shotgun" approach is far too vague to ascertain the involvement and capacity of the 5-HT$_3$ receptor system in any observed effect(s). Similarly, the systemic administration of 5-HT$_3$ receptor antagonists may be somewhat problematic, as both central (anti-nociceptive) and peripheral (pro-nociceptive) 5-HT$_3$ mediated mechanisms would be antagonized, thereby producing reductive or negligible effects. In light of this, the systemic administration of the 5-HT$_3$ receptor antagonist ondansetron was found to decrease the analgesic efficacy of tramad-ol, presumably by blocking central 5-HT$_3$ receptors within the dorsal horn that mediate anti-nociception (45). While animal studies have suggested that the differential antagonism of peripheral or central 5-HT$_3$ sites may be dependent upon dose and/or the route of parenteral administration (27, 36), such pharmacokinetic variables and their effects upon clinical pain reduction in humans remain unclear.

Alternatively, peripheral 5-HT$_3$ receptors that mediate a component of inflammatory pain may be effectively targeted through the use of local administration of currently available antagonists (e.g., ondansetron, granisetron, tropisetron). Using an experimental model of inflammatory pain, it has been shown that topical application of ondansetron in a lipophilic organogel produced dose-dependent reduction in both the nociceptive and inflammatory effects of intradermally injected capsaicin (46). Additionally, a pathologic elevation in intramuscular 5-HT may play a role in certain inflammatory myalgic conditions. This has been supported both by experimental studies in which local intramuscular injection of 5-HT produced pain and hyperalgesia in humans (47) and the demonstration of elevated muscular 5-HT in fibromyalgic and myofascial pain patients (48, 49). Taken together, such studies suggest that local use of selective 5-HT$_3$ receptor antagonists may have some utility in the clinical treatment of pain states that are mediated through 5-HT and 5-HT$_3$ dependent substrates (see also 50-53). The relative cost of these agents for such use may initially be prohibitive, however, in select cases of inflammatory pain states, such approaches may prove to be both time- and cost-effective.

As the field of 5-HT receptor biology expands, knowledge of 5-HT$_3$ and other 5-HT receptor-mediated mechanisms in pain and analgesia will provide inroads for the development of novel therapeutics in our armamentarium against pain. Together with the work of other groups, our ongoing studies are further exploring such possibilities.

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