Topical Review

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Diffuse noxious inhibitory controls and conditioned pain modulation: a shared neurobiology within the descending pain inhibitory system?

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1. Introduction

Descending pain inhibition is a key component of physiological and pathological pain processing.[70] Various neurotransmitter systems underlie different descending pain inhibitory pathways. Their anatomical and functional details have recently been revealed; thanks to new techniques that allow circuit tracing (virus-based tracing)[18,38] and circuit manipulation (chemogenetics and optogenetics)[93,95] with unprecedented precision.[37,47] Translation, however, remains challenging because the application of optogenetics and chemogenetics in humans faces substantial hurdles and because the more traditional pharmacological approaches would require that descending pain inhibition in humans and experimental animals involves the same transmitters and receptors. Prime examples of experimental paradigms assessing descending pain modulation in humans and animals are conditioned pain modulation (CPM) and diffuse noxious inhibitory controls (DNIC), respectively. Diffuse noxious inhibitory controls are measured as the inhibition of second-order wide dynamic range neurons (WDRs) by the application of a noxious stimulus outside the receptive field of the recorded neuron.[60] In CPM paradigms, this "pain-inhibits-pain" effect is assessed via the modulation of the perceived pain intensity caused by a noxious test stimulus by another noxious heterotopically applied "conditioning" stimulus.[98] Yet, few CPM studies offer mechanistic insights, making direct comparisons between DNIC and CPM mechanisms challenging. This topical review outlines preclinical evidence how various neurotransmitter systems contribute to descending pain inhibition and highlights those systems likely involved in DNIC. Indications for similar neurochemical processes in human CPM studies are discussed and synthesized with preclinical evidence, outlining gaps to be addressed by future studies.

2. Neurotransmitter systems involved in descending pain inhibition

The descending system engages various neurotransmitters to mediate antinociception in the spinal cord, including monoamines (mainly noradrenaline and serotonin). Antinociceptive effects of noradrenaline and spinal α2-adrenergic receptors are well-established.[8,97] The principal source of spinal noradrenaline is the locus coeruleus, and electrical/optogenetic stimulation of this brainstem area reduces pain sensitivity.[45,63] Chemogenetic activation of locus coeruleus' spinal projections reduces thermal sensitivity.[47] An antinociceptive function of serotonin has been demonstrated in some forms of descending inhibition, including stress-induced analgesia.[100] However, serotonin facilitates nociception in neuropathic pain models and direct optogenetic activation of serotonergic neurons in the ventral hind brain produces hypersensitivity.[16,94] Together, these results indicate both an inhibitory and a facilitatory role of serotonin in descending pain modulation, depending on the site of release and receptors engaged.[9]

Descending pain control also involves the opioidergic system, and microinjection of opioids into brain hubs of the descending pain inhibitory system, ie, the periaqueductal grey (PAG) and the rostroventromedial medulla (RVM), produces analgesia.[56] Although opioid receptors are distributed throughout the central nervous system (CNS), the strong analgesic effect of exogenous opioids is presumably mediated via descending pathways.[5,24] The opioid antagonist naloxone inhibits stimulation-produced analgesia,[1,19] supporting the importance of opioid signaling in endogenous pain inhibition. Enkephalin-containing descending projections from the RVM to the spinal cord produce analgesia upon activation, and enkephalin-containing spinal interneurons exist.[37,101] This indicates that exogenous and endogenous opioids can mediate pain inhibition at many levels of the pain axis.

Cannabinoids have also been linked to descending pain modulation. Although CB1 and CB2 receptors are found in the dorsal horn and spinal cannabinoid action reduces nociception,[82] cannabinoids also exert antinociceptive effects via supraspinal sites.[48,53,56,71] Endocannabinoids are involved in stress-induced analgesia through engagement of the PAG, and injection of cannabinoids into the PAG produces hypoalgesia,[48,62] making this area likely a main site of cannabinoid-mediated analgesia.

Finally, as principal mediators of fast inhibitory neurotransmission, γ-aminobutyric acid (GABA) and glycine are involved in numerous endogenous pain inhibitory processes[2,35] and likely interact with the descending pain inhibitory system at all levels of the CNS neuraxis.

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3. Which of these neurotransmitter systems play a role in diffuse noxious inhibitory controls?

Diffuse noxious inhibitory controls are mediated via descending pain inhibitory pathways.[6,10,22,56] Lesioning experiments have identified key CNS regions for DNIC, such as the ipsilateral dorsolateral funiculus and the medullary subnucleus reticulus dorsalis.[13,91] Of the neurotransmitter systems involved in descending pain inhibition, many affect DNIC, including noradrenaline and serotonin.[23,30] Noradrenergic and serotonergic neurons are rapidly activated by nociceptive stimuli, consistent with a role in DNIC.[65] Diffuse noxious inhibitory controls are prevented upon spinal delivery of α2-adrenoceptor antagonists, indicating that this specific receptor is a key mediator.[8] Of clinical relevance, DNIC are reduced in rodent models of chronic pain involving an altered balance between descending inhibition and facilitation, which can be restored by spinal blockade of serotonergic descending facilitation or spinal inhibition of noradrenaline reuptake.[8] Taken together, an involvement of noradrenaline and serotonin in DNIC is highly likely.

Besides the monoamines, endogenous opioid systems are likely involved in DNIC because systemic naloxone reduces the descending inhibition of WDRs by heterotopic noxious stimulation.[59] Further, enkephalin-like substance is released into the spinal cord upon noxious stimulation.[20] However, the precise involvement of opioids is complicated by observations that they inhibit DNIC when applied systemically.[58,61] These findings suggest that DNIC are partly mediated by endogenous opioids, but that the precise region of their release affects DNIC expression. For example, antagonizing κ-opioid receptor signaling in the right amygdala restores[74] and prevents the loss of[68] behavioral DNIC responses in stress-induced or experimental neuropathic pain models, respectively. Enkephalin-expressing spinal interneurons are directly inhibited by RVM GABAergic neurons and are activated through disinhibition to reduce mechanical sensitivity.[37] However, this mechanism only affects one sensory modality and is unlikely to be involved in DNIC, which have polymodal effects.[8,60]

Although endocannabinoids, GABA, and glycine mediate antinociception, their role in DNIC remains elusive. Spinal cannabinoid receptors exert a direct antinociceptive effect,[82] but to mediate DNIC, endocannabinoids would need to be produced throughout the entire spinal cord because these transmitters are produced on demand,[42] and DNIC globally inhibit spinal WDR firing. Roles of GABA and glycine in DNIC are difficult to assess pharmacologically because the fast inhibitory neurotransmitters are ubiquitous throughout the CNS.

4. Are these neurotransmitter systems similarly involved in conditioned pain modulation?

Analogous to DNIC, CPM depends on intact spinal and medullary structures because lesions in these areas impair CPM responses.[12,28,80] The preclinical evidence described above suggests a role of noradrenaline, serotonin, and endogenous opioids in DNIC and similar neurotransmitter systems need to be involved in CPM to infer a common mechanistic basis in both phenomena.

In human studies, one of few methods allowing direct investigation of a neurotransmitter's contribution to CPM is pharmacological manipulation, particularly in healthy volunteers with a presumably intact endogenous pain modulation (literature summary in **Table 1**). Kucharczyk et al. summarized pharmacological manipulations of DNIC, supporting an impact of noradrenergic, serotonergic, and opioidergic systems on DNIC.[54] In contrast to findings from animal experiments, pharmacological CPM studies question a critical role of noradrenaline in CPM. Systemic manipulations of α2-,[4,27,67] α1-,[27] or β-adrenoceptors[73] did not affect CPM except the application of one particular selective α2-adrenoceptor agonist, ie, dexmedetomidine.[4] In line with decreased DNIC after systemic administration in rats,[84] dexmedetomidine decreased CPM, supposedly because of a supraspinal effect of the α2 agonist inhibiting spinal noradrenaline release.[17] The contradictory findings in other studies, eg, systemic administration of the α1 agonist phenylephrine inhibiting DNIC[84] but not CPM,[27] remain to be clarified. Of potential relevance are noradrenaline effects on the cardiovascular system,[87] which affects pain responses,[14] including CPM.[21] Pharmacologically induced cardiovascular changes will interact with any direct pharmacological effect on CPM—a process that may differ in DNIC using anaesthetized animals.

Another means to modulate noradrenergic neurotransmission are noradrenaline reuptake inhibitors. Neurotransmitter-specific interpretations of available CPM studies are not possible because the applied pharmacological agents either included opioid action[64] or serotonin reuptake inhibition.[69] Serotonin and noradrenaline reuptake blockade[69] increased CPM in agreement with DNIC involving monoamines.[8] Whether this effect can be attributed to noradrenaline or serotonin remains to be disentangled.

Opioids have been extensively studied in human CPM studies using opioid receptor agonists[3,36,61,64,69,86] or antagonists.[15,33,36,40,44,52,72,85,96] By and large, the relevance of—exogenous and endogenous—opioids for CPM remains inconclusive. Predominantly, studies showed no effect of opioid receptor agonists[36,69,86] or antagonists[15,33,36,40,44,72,85] on CPM responses. The remaining results indicate reduced CPM after administration of opioid receptor agonists[61,64] *as well as* antagonists,[52,72,96] mirroring the conflicting results observed in DNIC described above. Increased CPM was observed only after prolonged (24-72 hours) administration of an opioid receptor agonist via transdermal patches.[3] It is unclear whether the ambiguous observations are due to variations in drug dosages/administrations, differences in the applied CPM paradigms,[49,76] varying affinities of the agents for different opioid receptor subtypes,[78] or else. Of note, akin to DNIC,[68,74] there is evidence for a role of opioid signaling within the right amygdala for CPM because higher basal μ-opioid receptor availability was associated with greater CPM effects.[75]

Investigations of cannabinoid function in CPM are similarly scarce as in DNIC. One study assessed CPM after exogenous administration of the cannabinoid nabilone, which had no effect.[79] It would be interesting whether interfering with the endocannabinoid system, eg, via antagonists, affected CPM.

The above-mentioned ubiquitous expression of GABA and glycine receptors in the CNS renders the examination of these neurotransmitters in pharmacological experiments difficult. A few human studies investigated how manipulating GABAergic inhibition affects CPM using nonselective[55,92] or subtype-selective[90] positive allosteric modulators (PAMs) of $GABA_A$ receptors. Conditioned pain modulation was not influenced by any of these compounds, suggesting that GABAergic neurotransmission is not key to CPM. No clinical data are available on glycine receptors because no glycine receptor modulators have so far been approved for use in humans.

For all neurotransmitters discussed, it needs to be considered that net zero effects on CPM after a systemic manipulation of a given system might reflect the sum of opposite nonzero effects. For example, $GABA_A$ receptor PAMs might act simultaneously at DNIC-relevant sites with a GABA-mediated antinociceptive (eg,

Table 1

Summary of existing pharmacological conditioned pain modulation studies in healthy volunteers.

Neurotransmitter system	Pharmacology	Pharmacological agent	N	Route of administration	TS/CS	Agent's effect on CPM	Reference (first author + year)
Noradrenaline	α1 agonist	Phenylephrine	20	Intravenous	Contact heat/hot water bath	No effect	Dayan,[27] 2018
	α2 agonist	Clonidine	20	Oral	Contact heat/hot water bath	No effect	Dayan,[27] 2018
	α2 agonist	Clonidine	40	Oral	Contact heat/cold water bath	No effect	Nahman-Averbuch,[67] 2016
	α2 agonist	Dexmedetomidine	10	Intravenous	Electrical/CO_2 laser	Reduced CPM	Baba,[4] 2012
	α2 antagonist	Yohimbine	20	Oral	Contact heat/hot water bath	No effect	Dayan,[27] 2018
	β-blocker	Propanolol	25	Oral	Pressure cuff/pressure cuff or cold water bath	No effect	Petersen,[73] 2018
Opioids	μ-OR agonist	Fentanyl	22	Transdermal patch	Pressure/cold water bath	Increased CPM	Arendt-Nielsen,[3] 2012
	μ-OR agonist	Fentanyl	16	Intravenous	Electrical/cold water bath	No effect	Okkerse,[69] 2017
	μ-OR agonist	Morphine	3 groups of 33/34	Intravenous	Contact heat/pressure cuff	No effect	France,[36] 2016
	μ-OR agonist	Morphine	9	Intravenous	Electrical/hot water bath	Reduced CPM	Le Bars,[61] 1992
	μ-OR agonist	Morphine	12	Oral	Contact heat/cold water bath	Reduced CPM	Martini,[64] 2015
	μ-OR agonist	Oxycodone	40	Oral	Contact heat/cold water bath	No effect	Suzan,[86] 2013
	μ-OR agonist (partial), κ-OR antagonist	Buprenorphine	22	Transdermal patch	Pressure/cold water bath	Increased CPM	Arendt-Nielsen,[3] 2012
	μ-, δ-, κ-OR antagonist	Naloxone	99	Intravenous	Contact heat/pressure cuff	No effect	Bruehl,[15] 2021
	μ-, δ-, κ-OR antagonist	Naloxone	6	Intramuscular injection	Contact heat/cold water bath	No effect	Edwards,[33] 2004
	μ-, δ-, κ-OR antagonist	Naloxone	3 groups of 33/34	Intravenous	Contact heat/pressure cuff	No effect	France,[36] 2016
	μ-, δ-, κ-OR antagonist	Naloxone	15	Intravenous	Pressure and pinprick/ intramuscular capsaicin	No effect	Graven-Nielsen,[40] 2002
	μ-, δ-, κ-OR antagonist	Naloxone	20	Subcutaneous injection	Pressure/pressure cuff	No effect	Hermans,[44] 2018
	μ-, δ-, κ-OR antagonist	Naloxone	6	Intravenous	Electrical/pressure cuff	No effect	Pertovaara,[72] 1982
			5	Intravenous	Contact heat and cold/ pressure cuff	Reduced CPM	
	μ-, δ-, κ-OR antagonist	Naloxone	20	Intravenous	Heat/ice bags	No effect	Sprenger,[85] 2011
	μ-, δ-, κ-OR antagonist	Naloxone	9	Intravenous	Electrical/hot water bath	Reduced CPM	Willer,[96] 1990
	μ-, δ-, κ-OR antagonist	Naltrexone	33	Oral	Contact heat/cold water bath	Reduced CPM	King,[52] 2013
GABA	Nonselective PAM of $GABA_A$ receptors	Clobazam	16	Oral	Pressure/cold water bath	No effect	Vuilleumier,[92] 2013
	Nonselective PAM of $GABA_A$ receptors	Clonazepam	16	Oral	Pressure/cold water bath	No effect	Vuilleumier,[92] 2013
	Nonselective PAM of $GABA_A$ receptors	Lorazepam	20	Oral	Electrical/contact heat	No effect	Kunz,[55] 2006
	Subunit-selective PAM of $GABA_A$ receptors	PF-06372865	20	Oral	Electrical/cold water bath	No effect	Van Amerongen,[90] 2019
	Synthetic cannabinoid	Nabilone	17	Oral	Contact heat/cold water bath	No effect	Redmond,[79] 2008
Mixed	SNRI	Imipramine	16	Oral	Electrical/cold water bath	Increased CPM	Okkerse,[69] 2017
	NRI + μ-OR agonist	Tapentadol	12	Oral	Contact heat/cold water bath	No effect	Martini,[64] 2015

Literature was reviewed from inception to April 12, 2021 using PubMed and EMBASE databases. Only studies assessing effects after acute administration of pharmacological agents (not after treatment over multiple days) in healthy volunteers were reviewed. Pharmacological agents in all these studies were administered systemically. Studies combining CPM with other interventions (eg, transcranial stimulations) were not included. One study using naloxone[53] was not included as "opioid antagonist" study because naloxone was applied after morphine and the effects consequently mirror reversal of opioid agonism rather than pure opioid receptor blockade.

CPM, conditioned pain modulation; CS, conditioning stimulus; GABA, γ-aminobutyric acid; NRI, noradrenaline reuptake inhibitor; OR, opioid receptor; PAM, positive allosteric modulator; SNRI, serotonin–noradrenaline reuptake inhibitor; TS, test stimulus.

the spinal dorsal horn[32]) or pronociceptive function (eg, the RVM[31]).

5. Synthesis

Over the past decades, the knowledge about descending pain inhibitory controls in preclinical models has advanced considerably, including the function of distinct neurotransmitters. This knowledge might be translated to humans through experimental paradigms applicable across species, such as DNIC in rodents and CPM in humans. However, as highlighted throughout this review, further investigations are needed to clarify contradictory results within both phenomena and to validate proposed commonalities in their underlying mechanisms.

One open question regarding the mechanisms of DNIC concerns the precise functional identities of WDRs.[29] It is not clear whether these are inhibitory or excitatory, local or projection neurons,[57] and they have not been assigned to any molecular classes of neurons defined using single-cell RNA sequencing.[43] It is further unclear how inhibition of WDRs, found within lamina V of the dorsal horn, translates into changes in pain perception because the nociceptive-specific region of the spinal cord is considered to be within superficial laminae.[89] The precise identities of descending fibers mediating DNIC are also elusive.

Descending pathways mediating DNIC would exhibit 4 features: (1) be engaged by noxious but not innocuous stimuli, (2) have whole-body receptive fields, (3) project directly to the spinal cord, and (4) be capable of suppressing WDR firing when activated.[57] Fundamentally, the exact mechanism of WDR inhibition is unknown and could involve direct postsynaptic inhibition, engagement of inhibitory spinal circuits, or presynaptic inhibition of the nociceptive afferent input onto WDRs.

Several recent technological developments could help address these questions. Neurochemical sensors can be used to assess the amount of neurotransmitter released in the spinal cord during heterotopic noxious stimuli to identify neurotransmitter systems involved in DNIC.[77] Retrograde viral tracing techniques can be used to introduce genetic material into neurons via their axon terminals in the spinal cord.[47,88] Combined with cre-expressing mouse lines, retrograde transduction enables access to and functional manipulation of specific descending systems and can establish causal relationships between descending systems and DNIC through gain-of-function and loss-of-function experiments.[37,101] Several related technologies for the capture and reactivation of neurons engaged in different behavioral pain states would facilitate their study and potentially identify novel CNS regions and neurons associated with DNIC.[41,81,83]

Efforts to further advance the understanding of DNIC mechanisms should be paralleled by mechanistic investigations of the human counterpart, CPM. One major challenge is the sheer abundance of CPM methods hindering comparability, meta-analytical approaches, and generalizability. Current expert consensus recognizes the difficulty of defining a gold-standard CPM assessment because none of the available paradigms seems superior to others.[99] Settling on *one* gold-standard paradigm might even not be the optimal solution because distinct paradigms allow investigation of CPM effects on different nerve fiber classes, eg, deep vs superficial fibers. Developing standardized protocols for *multiple* test stimuli/conditioning stimuli combinations would improve comparability between studies. For mechanism-oriented studies, CPM paradigms would benefit from: (1) including a nonpainful control conditioning stimulus (SHAM) to disentangle real CPM effects from repeated-measures or antinociceptive SHAM effects,[26] (2) assessing parallel *and* sequential CPM effects to better understand the respective outcomes, and (3) monitoring cardiovascular changes, particularly when using the most frequently applied[50] conditioning stimulus, ie, a cold water bath, originally designed to assess cardiovascular reactivity.[46] Finally, CPM effects are mainly explained by interindividual differences other than age, sex, and conditioning stimulus intensity,[39] and their origin (eg, psychological or genetic) requires further investigation.

Advanced imaging methods and integrative approaches could improve the understanding of CPM mechanisms. For example, simultaneous functional magnetic resonance imaging (MRI) of the brainstem and spinal cord[11] during a CPM paradigm can help to dissociate supraspinal vs spinal changes in response to CPM, particularly at higher spatial resolution provided by ultra-high field MRI at 7 T. Another perspective on dynamic neuronal processes is offered by functional MR spectroscopy (MRS)[66] and tracking glutamate or GABA alterations during CPM could elucidate associated excitatory/inhibitory processes. Combining functional MRI and functional MRS might allow examining interactions between neurochemical and neurophysiological processes,[51] including those underlying CPM.

Integrating such advanced MR methods with pharmacological manipulations of specific neurotransmitter systems would clarify CPM processes in even more detail. More targeted pharmacological manipulations are favorable for more specific mechanistic interpretation. Multimodal approaches are particularly warranted because behavioral outcomes of CPM are highly variable[34] and may lack sensitivity to detect subtle effects. For instance, alterations in neuronal processing detected by functional MRI during CPM after naloxone administration were not reflected in recorded behavioral outcomes.[85]

Finally, DNIC- and CPM-specific knowledge can be ultimately synthesized in studies applying uniform experimental designs in rodents and humans. Besides identical test and conditioning stimuli,[25] similar pharmacological manipulations of specific neurotransmitter systems are conceivable using identical pharmacological agents and routes of administration. Also, DNIC assessments in behaving, not-anaesthetized, animals, termed "descending control of nociception,"[7] might be more relevant for direct comparisons with CPM.

In summary, further translational and back-translational efforts are needed to provide details of the neuronal circuitry and transmitter systems responsible for descending pain inhibition across species.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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