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Review

Targeting the cholinergic system as a therapeutic strategy for the treatment of pain

Philip G. Jones*, John Dunlop

Neuroscience Discovery Research, Wyeth Research, CN8000, Princeton, NJ 08543, USA

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Abstract

Acetylcholine mediates its effects through both the nicotinic acetylcholine receptors (ligand-gated ion channels) and the G protein-coupled muscarinic receptors. It plays pivotal roles in a diverse array of physiological processes and its activity is controlled through enzymatic degradation by acetylcholinesterase. The effects of receptor agonists and enzyme inhibitors, collectively termed cholinomimetics, in antinociception/analgesia are well established. These compounds successfully inhibit pain signaling in both humans and animals and are efficacious in a number of different preclinical and clinical pain models, suggesting a broad therapeutic potential. In this review we examine and discuss the evidence for the therapeutic exploitation of the cholinergic system as an approach to treat pain.

Keywords: Pain; Muscarinic receptor; Nicotinic receptor; Acetylcholine; Acetylcholinesterase

1. Introduction

Acetylcholine mediates its effects through both the nicotinic acetylcholine receptors (ligand-gated ion channels) and the G protein-coupled muscarinic receptors. It plays pivotal roles in a diverse array of physiological processes and its activity is controlled through enzymatic degradation by acetylcholinesterase. The effects of receptor agonists and enzyme inhibitors, collectively termed cholinomimetics, in antinociception/analgesia are well established. These compounds successfully inhibit pain signaling in both humans and animals and are efficacious in a number of different preclinical and clinical pain models (see Table 1), suggesting a broad therapeutic potential. In this review we examine and discuss the evidence for the therapeutic exploitation of the cholinergic system as an approach to treat pain.

2. Mechanisms of cholinomimetic analgesia

A major site of action for cholinomimetics in analgesia is the spinal cord. Intrathecal cholinergic agents cause antinociception by mimicking the release of acetylcholine (ACh) from the spinal cholinergic nerves. Painful stimuli are known to increase ACh in the spinal cord, as seen in the CSF of anesthetized sheep following noxious stimulation (Eisenach et al., 1996). This ACh is released from the cholinergic interneurons in the dorsal horn, which are activated by the inhibitory descending noradrenergic and serotonergic pain modulatory pathways. In support of this, the antinociceptive effects of the α2-adrenergic agonist clonidine can be blocked by atropine (Zhuo and Gebhart, 1990, 1992; Detweiler et al., 1993). More recently, Duflo et al. (2005) have presented data which indicate that spinal muscarinic receptors mediate the antinociceptive effects of clonidine in a model of post-operative pain, in which hyperalgesia occurs following incision of the plantar surface of the rat hind paw (Brennan et al., 1996). Furthermore, the actions of α2-adrenergic agonists in the sheep are enhanced by intrathecal cholinesterase inhibitors in the post surgical period (up to 5 days post surgery) (Bouaziz et al.,

* Corresponding author. Present address: Discovery Research, Sepracor Inc., 84 Waterford Drive, Marlborough, MA 01752, USA. Tel.: +1 508 787 4532; fax: +1 508 490 5454.
E-mail address: philip.jones@sepracor.com (P.G. Jones).

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Table 1
Summary of the actions of cholinomimetics in animal models of pain

Target	Compound	Pain model	Reference(s)
AChE	Neostigmine	Intraplantar capsaicin, noxious cold, post-incisional, radiant heat, Stz-induced diabetes, SNL	Bouaziz et al., 1995; Chen et al., 2001; Eisenach et al., 1996; Hood et al., 1997; Lavand'homme and Eisenach, 1999
mAChR	WAY-132983	PPQ, SNL, FCA, PGE2, Post-incisional, MIA	Sullivan et al., 2005
	Bethanecol	Tail-flick, post-incisional	Prado and Segalla, 2004
	Oxotremorine	Tail-flick, hotplate, acetic-acid writhing	Gomeza et al., 1999; Sheardown et al., 1997
	Arecaidine	Orofacial pain	Dussor et al., 2004
	Vedaclidine	Hot-plate, tail flick, intra-plantar capsaicin	Shannon et al., 2001
nAChR	ABT-594	Hot box, formalin hot plate, cold plate, PPQ, chemotherapy	Bannon et al., 1998a,b; Decker et al., 1998; Lynch et al., 2005
	Epibatidine/analogues	Tail flick, hot plate, intracapsular carageenan	Carroll et al., 2002; Lawand et al., 1999; Nishiyama et al., 2003
	RJR-2403	SNL	Lavand'homme and Eisenach, 1999
	Dimethylphenyl-piperazinium	Tail-flick, post-incisional	Prado and Segalla, 2004

1995), consistent with the reported increase in spinal ACh (Eisenach et al., 1996).

The neurons in lamina II of the spinal cord have an important role in pain transmission since they receive excitatory and inhibitory signals from the periphery, from spinal interneurons and also from supraspinal regions. Li et al. (2002) in a detailed study, reported that acetylcholine attenuated miniature excitatory post-synaptic currents (mEPSCs) and evoked EPSCs (eEPSCs) in the substantia gelatinosa. These eEPSCs were sensitive to CNQX indicating that they are mediated by glutamate, the major excitatory amino acid in the brain and spinal cord. Together, these observations suggest that the actions of ACh are to reduce the release of glutamate. The demonstration that ACh can also reduce mEPSCs in the presence of tetrodotoxin suggests the inhibition of glutamate release is via a direct effect mediated by presynaptic mAChRs (Li et al., 2002). These authors also confirm the presence of presynaptic M2 receptors by immunohistochemical studies (Li et al., 2002).

In addition to decreasing excitatory transmission, acetylcholine was also demonstrated to increase the frequency of miniature inhibitory post-synaptic currents (mIPSCs) in a spinal cord slice preparation in rats, indicating a presynaptic stimulatory effect on the release of GABA (Li et al., 2002). GABA acting through $GABA_B$ receptors inhibits mEPSCs in the rat spinal cord (Iyadomi et al., 2000) and interestingly, the effects of ACh on glutamate release are, in part, sensitive to the $GABA_B$ receptor antagonist CGP55845. This suggests a second indirect mechanism through which ACh can inhibit excitatory neurons by increasing the inhibitory GABAergic tone, which then reduces glutamate release (Li et al., 2002). Supporting these observations are reports that the analgesic and antiallodynic actions of intrathecal ACh or neostigmine in diabetic rats is inhibited by CGP55845 (Li et al., 2002; Chen and Pan, 2003). Species specific effects may exist since in contrast to the data obtained in the rat, in mice the M3 receptor may play a greater role in GABA release as oxotremorine-M increased inhibitory currents in the spinal cord of M2/M4 knockout mice (Zhang et al., 2006). Activation of the M2/M4 receptors in the absence of M3 is reported to reduce GABAergic transmission. However, the significance of this observation is questioned though by the demonstration that oxotremorine-M fails to evoke any antinociceptive activity in M2/4 knockout mice, as described later (Duttaroy et al., 2002).

Further evidence in support of increased inhibitory transmission comes from the demonstration that ACh increased the release of the inhibitory transmitter glycine, from spinal cord interneurons in the rat (Wang et al., 2006). The absence of M1 in the spinal cord and the observations that this effect is still apparent in pertussis-toxin treated rats would suggest that it is mediated through the M3 receptor. The attenuation seen with 4-DAMP, a muscarinic antagonist with high affinity for the M3 receptor, would support this although the selectivity is questionable at the concentrations used.

Although care should be exercised in the interpretation of both pharmacological studies with the currently available ligands and studies using constitutive receptor knockouts, there is strong evidence that activation of muscarinic receptors in the spinal cord results in an increased release of inhibitory transmitters along with a decrease in the release of excitatory transmitters, and this in part mediates their antinociceptive effects.

The spinal cholinergic system also plays a role in the actions of opiates since spinally administered atropine can reduce the analgesic effects of systemically administered morphine in rats (Chiang and Zhuo, 1989). Furthermore, neostigmine enhances the ability of morphine to reduce thermal pain in rats (Eisenach and Gebhart, 1995). The study of Hood et al. (1997) confirms this interaction of the cholinergic and opioid system in the human. Alfentanil was reported to increase ACh concentrations in the CSF and its analgesic effect was enhanced by neostigmine (Hood et al., 1997).

A supraspinal component to nociception/antinociception also exists for the cholinergic system. Injection of carbachol into the dorsal periaqueductal gray (PAG) increases the withdrawal latency in the rat tail flick assay (Guimaraes and Prado, 1994; Guimaraes et al., 2000). Furthermore, a decrease in vocalization both during and following a noxious stimulus is also seen on carbachol administration (Guimaraes et al., 2000). The tail flick and vocalization during stimulus were inhibited by mecylamine indicating that they are mediated through the nicotinic receptors. The tail flick is however a spinal response and