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PAIN SENSITIVITY FOLLOWING LOSS OF CHOLINERGIC BASAL FOREBRAIN (CBF) NEURONS IN THE RAT

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Abstract—Flexion/withdrawal reflexes are attenuated by spinal, intracerebroventricular (ICV) and systemic delivery of cholinergic agonists. In contrast, some affective reactions to pain are suppressed by systemic cholinergic *antagonism*. Attention to aversive stimulation can be impaired, as is classical conditioning of fear and anxiety to aversive stimuli and psychological activation of stress reactions that exacerbate pain. Thus, in contrast to the suppressive effects of cholinergic agonism on reflexes, pain sensitivity and affective reactions to pain could be attenuated by *reduced* cerebral cholinergic activation. This possibility was evaluated in the present study, using an operant test of escape from nociceptive thermal stimulation (10 °C and 44.5 °C) before and after destruction of basal forebrain cholinergic neurons. ICV injection of 192 IgG-saporin produced widespread loss of basal forebrain cholinergic innervation of the cerebral cortex and hippocampus. Post-injection, escape from thermal stimulation was decreased with no indication of recovery for upto 19 weeks. Also, the normal hyperalgesic effect of sound stress was absent after ICV 192-sap. Effects of cerebral cholinergic denervation or stress on nociceptive licking and guarding reflexes were not consistent with the effects on operant escape, highlighting the importance of evaluating pain sensitivity of laboratory animals with an operant behavioral test. These results reveal that basal forebrain cholinergic transmission participates in the cerebral processing of pain, which may be relevant to the pain sensitivity of patients with Alzheimer's disease who have prominent degeneration of basal forebrain cholinergic neurons.

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Abbreviations: CBF, cholinergic basal forebrain; HDB, horizontal diagonal band; ICV, intracerebroventricular; MS, medial septum; NBM, nucleus basalis of Meynert; VDBB, vertical diagonal band of Broca.

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INTRODUCTION

Cholinergic agonists are antinociceptive at spinal levels (Jones and Dunlop, 2007; Bartolini et al., 2011), reducing clinical pain in humans (Lauretti and Lima, 1996; Hood et al., 1997), and intrathecal administration of cholinergic agonists attenuates flexion/withdrawal reflexes of laboratory animals (Yaksh et al., 1985; Hartvig et al., 1989; Gillberg et al., 1990; Bouaziz et al., 1995; Eisenach and Gebhart, 1995). Also, suppression of spinal reflexes results from systemic muscarinic or nicotinic agonism (Hendershot and Forsaith, 1959; Harris et al., 1969; Ireson, 1970; Pedigo et al., 1975; Yaksh et al., 1985; Gower, 1987; Hartvig et al., 1989; Bartolini et al., 1992; Rao et al., 1996; Thor et al., 2000; Abelson and Hoglund, 2002; Prado and Dias, 2009) and acetylcholinesterase antagonism (Hendershot and Forsaith, 1959; Harris et al., 1969; Ireson, 1970; Cozanitis et al., 1983; Hartvig et al., 1989; Abram and Winne, 1995; Hood et al., 1995, 1997; Lauretti and Lima, 1996; Buerkle et al., 1998; Hwang et al., 2001; Nicolodi et al., 2002; Mojtahedin et al., 2009; Prado and Dias, 2009; Kimura et al., 2013; Prado and Goncalves, 1997; Yaksh et al., 1985; Yoon et al., 2003). Furthermore, depression of spinal reflexes is enhanced when cholinergic agents act in support of other transmitters (e.g., opioids: (Pedigo et al., 1975; Chiang and Zhuo, 1989; Eisenach and Gebhart, 1995; Hood et al., 1997); noradrenaline: (Zhuo and Gebhart, 1990; Detweiler et al., 1993; Bouaziz et al., 1995; Eisenach, 1999; Duflo et al., 2005); and GABA: (Kimura et al., 2013).

The above-cited literature has generated remarkably consistent results. However, studies of cholinergic agonist and antagonist effects on the nociceptive sensitivity of animal subjects have focused exclusively on spinal mechanisms, evaluating flexion/withdrawal reflex responses to radiant heat (tail flick), contact heat (hot plate), mechanical stimulation, tail immersion, or chemical injection (formalin). These reflex tests do not assess effects of pharmacological agents on pain intensity or its motivating properties, which depend upon cerebral activation by nociceptive input. Alternatively, pain sensitivity and its motivating properties can be evaluated by monitoring escape responses to nociceptive input (Vierck, 2005, 2006a; Vierck et al., 2008a; Vierck and Yezierski, 2015). Consciously

motivated responses that escape or avoid nociceptive stimuli reveal characteristics of pain sensations that require cerebral processing of input from spinothalamocortical neurons. Assessment of behavioral responses that reflect cerebral processing of pain intensity is crucial for studies involving systemic or intracerebral delivery of compounds with putative anti- or pro-nociceptive effects (Vierck et al., 2013).

Effects of cholinergic agents on operant avoidance responses of laboratory animals have been studied extensively with the intention to reveal deficits in learning. For example, during training on passive avoidance tasks, cholinergic antagonists have been administered before trials in which animals receive electrical stimulation when they enter one of two compartments of a shuttle box. According to the spinal actions of cholinergic antagonists, sensitivity to electrocutaneous shock is increased by cholinergic antagonism, and avoidance should be enhanced, but learning not to enter the shock compartment is impaired under the influence of systemic scopolamine (Bohdanecky and Jarvik, 1967; Gruber et al., 1967; Lo et al., 1982; Elrod and Buccafusco, 1988; Henzi et al., 1990; Wilson and Cook, 1994; Garcia-Alloza et al., 2006) or following intracerebroventricular (ICV) injection of 192 IgG-saporin (192-sap) that destroys cholinergic basal forebrain (CBF) neurons. 192-sap eliminates cholinergic input to almost all the cerebral cortex and the hippocampus (Wiley et al., 1995; Zhang et al., 1996; Garcia-Alloza et al., 2006). Avoidance deficits and impaired maze learning by scopolamine (Garcia-Alloza et al., 2006; Hodges et al., 2009; Doguc et al., 2012) or ICV 192-sap (Wrenn et al., 1999) are consistent with evidence from human studies that cerebral cholinergic transmission supports new memory formation when intact and impairs new memory formation when reduced (Ellis et al., 2006; Rokem and Silver, 2013; Wallace and Bertrand, 2013). However, the results of avoidance tests also are consistent with the possibility that systemic cholinergic antagonism or basal forebrain cholinergic denervation reduces pain sensitivity by cerebral effects that differ from spinal effects.

Clearly, the cerebral actions of cholinergic agonists and antagonists are not restricted to influences on learning and memory but also include effects on attention, arousal and emotionality, which could impact pain sensitivity in ways distinct from the spinal actions of these agents. (1) Some investigations have implicated attention deficits as responsible for impaired learning following administration of cholinergic antagonists (Furey, 2011; Thienel et al., 2009; Klinkenberg and Blokland, 2010). A distinction is made between top-down attention that is directed by prior experiences and bottom-up attention to stimuli which are innately of interest (e.g., stimuli eliciting pain). In either case, attention can be improved by cholinergic agonists, enhancing the signal-to-noise ratio, and attention is impaired by cholinergic antagonists (Sarter et al., 2005; Thienel et al., 2009; Klinkenberg and Blokland, 2010; Klinkenberg et al., 2011) and by lesions of the CBF using 192-sap (Waite et al., 1999). (2) Fear conditioning, a type of learning particularly dependent upon cholinergic innervation of limbic system structures (Graeff et al., 1980; Phillips and LeDoux, 1992; Schiller et al., 2008; Santini et al., 2012), is impaired by administration of cholinergic antagonists (Mollenauer et al., 1973, 1976, 1979; Anagnostaras et al., 1999; Zelikowsky et al., 2013). Anxiety and fear of pain are predictive of exaggerated pain and pain sensitivity over time (Turk and Wilson, 2010; Parr et al., 2012; Belfer et al., 2013; Castillo et al., 2013; Zale et al., 2013). (3) Another negative mood state, depression, is often associated with chronic pain, is enhanced by cholinergic agonists and is ameliorated by cholinergic antagonists (Janowsky et al., 1974; Dilsaver, 1986; Furey et al., 2010). (4) Cholinergic antagonism shifts electroencephalographic activity toward high-voltage, slow rhythms (Ebert and Kirch, 1998) and produces drowsiness (Longo, 1966). Accordingly, scopolamine has been utilized as an amnestic presurgical sedative (Conner et al., 1977; Kamboj and Curran, 2006). (5) Psychological stress has been shown in numerous experiments to attenuate nociceptive reflexes (e.g., (Porro and Carli, 1988; Bodnar, 1990; Yamada and Nabeshima, 1995), but pain sensitivity, as assessed by operant escape, is enhanced by stress (King et al., 2003, 2007; Marcinkiewcz et al., 2009). Also, chronic stress is a risk factor for development of chronic pain (Vierck, 2006b; Runnals et al., 2013). Cholinergic agonists enhance physiological indices of psychological stress (Janowsky et al., 1983; Finkelstein et al., 1985; Gilad et al., 1985; Dilsaver et al., 1986; Lai et al., 1986).

Impaired attention, reduced anxiety, fear, depression and an attenuated stress response could reduce pain sensitivity and affect, particularly in combination. Accordingly, the present investigation utilized an operant escape task to evaluate the pain sensitivity of rats before and after selective reduction of cerebral cholinergic innervation by ICV injection of 192-sap. The *a priori* hypothesis was that loss of basal forebrain cholinergic neurons would decrease sensitivity to nociceptive thermal stimulation and would attenuate the hyperalgesia that normally follows psychological stress by exposure to loud sound.

EXPERIMENTAL PROCEDURES

Female Long–Evans hooded rats were acclimated to the vivarium at 8 months of age and were maintained in pairs in standard shoe box cages. Food and water were provided *ad libitum*. Weights were recorded weekly to monitor general health. All experimental procedures were approved by the University of Florida Institutional Animal Care and Utilization committee and were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No 80-23, revised 1996). All efforts were made to minimize the number of animals used and their suffering.

Escape training and testing

The escape apparatus is divided into two ventilated compartments by a hanging partition with an opening