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ITCH ELICITED BY INTRADERMAL INJECTION OF SEROTONIN, INTRACISTERNAL INJECTION OF MORPHINE, AND THEIR SYNERGISTIC INTERACTIONS IN RATS

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Abstract—We used the cheek model of itch and pain in rats to determine the dose–response relationships for intradermal injection of serotonin and α methylserotonin on scratching behavior. We also determined the dose-related effects of intracisternally injected morphine on scratching, effects that were greatly reduced by administration of the opiate antagonist naloxone. We then examined the interactions of intradermal injection of serotonin and intracisternal injection of morphine on scratching and found that the two procedures act synergistically to increase itch. These results suggest that morphine applied to the CNS is capable of producing itch and greatly increasing itch originating in the skin (hyperknesis).

Key words: hyperknesis, itch, serotonin, morphine, intracisternal injection.

INTRODUCTION

The unpleasant sensations of itch and pain are each mediated by nociceptive neurons (Davidson and Giesler, 2010; Akiyama and Carstens, 2013; LaMotte et al., 2013), yet the sensations are perceived as distinct and induce very different behavioral responses. For example itch causes the desire to scratch and pain causes discomfort which results in guarding or withdrawal from a noxious stimulus. The use of animal models that allow differentiation of itch- versus pain-related behaviors are important in understanding the sensory effects of potential pruritogens or algogens. Until recently, such a model in rodents was not available. However, Shimada and LaMotte (2008) showed that pruritogens and algogens elicit distinct behaviors when applied to the cheek of mice; intradermal injection of histamine in the cheek elicited scratching with the hindlimb whereas capsaicin elicited wiping with the forelimb. Both behaviors were directed to the site of injection. This distinction between itch-evoked scratching and pain-evoked wiping has been replicated using an array of other pruritogens and algogens in mice (Akiyama et al., 2010; Wilson et al., 2011) and rats (Klein et al., 2011; Spradley et al., 2012).

Serotonin is one the most effective pruritogens in the cheek model in rats (Klein et al., 2011). Application of serotonin to the skin can also cause itch in humans (Fjellner and Hägermark, 1979; Weisshaar et al., 1997; Thomsen et al., 2001; Hosogi et al., 2006; Rasul et al., 2012). In several conditions of chronic itch, including allergic contact dermatitis and atopic dermatitis, the skin of patients exhibits increased levels of serotonin (Lundeberg et al., 1999; Soga et al., 2007). Serotonin can also elicit pain in humans (Schmelz et al., 2003). Accordingly, when applied to the rat cheek, serotonin elicits scratching with the hindlimb as well as some wiping with the forelimb (Klein et al., 2011). It has been suggested that serotonin may cause itch in humans via serotonin-induced release of histamine from mast cells (Weisshaar et al., 1997), although administration of antihistamines failed to result in a significant reduction of serotonin-induced itch compared to placebo treatment (Hosogi et al., 2006). In rats, mast cells release large amounts of serotonin (Gustafsson, 1980; Graziano, 1988; Purcell et al., 1989). In addition, topical application of serotonin activated a subpopulation of polymodal nociceptive dorsal root ganglion (DRG) neurons with C axons and the duration of the responses of some of the recorded fibers matched the duration of scratching or biting evoked by the same stimulus in awake rats (Hachisuka et al., 2010), suggesting that such C fibers play a role in production of itch. Intradermal injection of serotonin was also shown to elicit scratching of the nape of the neck and activity in lumbar dorsal horn neurons (Jinks and Carstens, 2002). In addition, we (Moser and Giesler, 2014) recently reported that intradermal injection of a dose of serotonin that produced scratching for more than 30 min in rats (Klein et al., 2011) activated pruriceptive trigeminothalamic tract neurons with receptive fields on the cheek for a similar period of time. Together, these data support the use of serotonin as a peripheral pruritogen.

In addition to pruritogens which cause itch via activation of peripheral nociceptors, there are a number of agents which cause itch-related behaviors when administered to the CNS in animal models (Koenigstein,

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Abbreviations: α-Me-5HT, α-methylserotonin maleate salt; DRG, dorsal root ganglion.

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1948; Thomas and Hammond, 1995; Lee et al., 2003; Sun and Chen, 2007; Su and Ko, 2011; Mishra and Hoon, 2013). Morphine is one of the most frequently studied agents of this type. It is commonly prescribed for relief from pain, but side-effects, including severe itch, can limit the amount that be administered, and thus the effectiveness of morphine for producing analgesia. Opioid-induced pruritus is often localized to facial regions of patients (Scott et al., 1980; Collier, 1981; Baraka et al., 1982; Bromage et al., 1982), suggesting the value of using the rodent cheek model of itch to study this phenomenon. The highest incidence of opioid-induced pruritus in human patients (20–100%) occurs following intrathecal administration (Baraka et al., 1982; Bromage et al., 1982; Ballantyne et al., 1988; Szarvas et al., 2003; Ganesh and Maxwell, 2007). Intracisternal injection of morphine in rats causes robust body and facial scratching (Lee et al., 2003) as does injection of morphine within the spinal trigeminal nucleus (Thomas and Hammond, 1995). In addition, we have found that application of morphine (200 nM) to the dorsal surface of upper cervical segments and the lower medulla activates pruriceptive trigeminothalamic tract neurons (Moser and Giesler, 2013). Thus, the rat trigeminal system appears to be of considerable potential value for studies of the mechanisms underlying opioid-induced pruritus.

Morphine and other pharmacological agents which act at μ-opioid receptors appear to modulate itch caused by other stimuli. Spradley et al. (2012) showed that the μ-opioid receptor antagonist naltrexone reduced scratching caused by facial application of pruritogens, whereas morphine reduced wiping caused by algogens in rats. This finding suggests that μ-opioid receptor activation has opposite effects on processing of itchy versus painful stimuli applied to the cheek. Opioids such as morphine likely also play a role in producing other itch-related sensory phenomena such as hyperknesis (increased itch caused by pruritogens) (Fjellner and Hägermark, 1982; Onigbogi et al., 2000) and alloknesis (itch caused by innocuous mechanical stimuli that normally do not cause itch) (Koenigstein, 1948; Heyer et al., 2002).

We used the cheek model of itch to examine the relationship of itch induced by intradermal injection of serotonin and intracisternal injection of morphine in rats. We report that activation of opioid receptors by intracisternal injection of morphine increases scratching responses to intradermal injection of serotonin and that this interaction is super-additive or synergistic.

EXPERIMENTAL PROCEDURES

Animals

Adult male Sprague–Dawley rats (200–300 g) were used according to protocols approved by the Institutional Animal Care and Use Committee at the University of Minnesota. Animals arrived at the university at least 1 week prior to testing. At least two days prior to testing, animals underwent light anesthesia with isoflurane (3% in 100% oxygen, < 5 min) and their cheeks and necks were shaved.

Intradermal injections into the cheeks

For intradermal injections in the cheeks, awake rats were gently restrained using a transparent flexible plastic cone. One experimenter held an animal within the cone while another experimenter performed the injection. Drugs for intradermal injection included serotonin creatinine sulfate complex (9–180 μg, Sigma, St. Louis, MO), α-methylserotonin maleate salt (α-Me-5HT; 3–30 μg, Sigma), or 0.9% normal saline vehicle. Drugs were injected in a 10-μl volume using a 28-gauge hypodermic needle inserted into the skin on the cheek below the eye and caudal to the vibrissal pad. Intradermal injection was confirmed by observation of a small bleb at the injection site.

Intracisternal injections

Rats were lightly anesthetized using isoflurane (3%, < 5 min) and intracisternal injections were performed according to Appel and Van Loon (1986). One experimenter held the animal's head between the thumb and forefinger of the left hand behind the animal's ears and suspended the animal with the right hand, maximally opening the foramen magnum at the base of the skull. Another experimenter performed the injection by inserting a 25-gauge 5/8-inch needle attached to a 100-μl Hamilton microsyringe through the shaved skin on the back of the neck until the needle came into perpendicular contact with the occipital bone. The needle was then moved ventrally, depressing muscles in the neck, until it could be inserted under the atlanto-occipital membrane through the foramen magnum into the cisterna magna. Drugs for intracisternal injection included morphine sulfate (0.01–333.0 μg, Sigma) or 0.9% normal saline vehicle. Drugs were injected in a 20-μl volume. We wished to evaluate the effectiveness of our methods for producing injections into the cisterna magna. We were reluctant to inject a solution containing morphine and a dye since it was possible that the dye itself might affect the CNS or the diffusion of the morphine. The following approach was used: 15 rats that had been used in intracisternal injection protocols received an injection of methylene blue (2%, 20 μl) using the same injection methods just prior to being euthanized and perfused with 0.9% normal saline. The presence of blue dye under the dura of the caudal medulla and/or rostral cervical spinal cord was used to verify an intracisternal injection. Intraperitoneal (i.p.) or subcutaneous (s.c.) injections of morphine (1 mg/kg, i.p.) or naloxone (1 mg/kg, i.p.; 0.5 mg/kg, s.c.) were administered 10 min prior to subsequent intradermal and/or intracisternal injections. When intradermal serotonin and intracisternal morphine were delivered in combination, intracisternal injections occurred within 5 min prior to intradermal injections.

Behavioral protocol

One week before testing began, animals were habituated for 60 min/day for 5 days to an acrylic chamber (15 × 15 × 23 cm) located in a lighted room. On the following test days, before intradermal or intracisternal injection, animals were habituated for 15 min before