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The histaminergic H_1, H_2, and H_3 receptors of the lateral septum differentially mediate the anxiolytic-like effects of histamine on rats' defensive behaviors in the elevated plus maze and novelty-induced suppression of feeding paradigm

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HIGHLIGHTS

- We investigated the effects of histamine in two rat models of anxiety.
- Lateral septal histamine increased rats' open arm activity and reduced neophagia.
- The effects of histamine on open arm activity were reversed by an H3 antagonist.
- The effects of histamine on neophagia were reversed by H1 and H2 antagonists.
- The contribution of specific histamine receptors to anxiety is test-specific.

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ABSTRACT

The neural histaminergic system is involved in a wide range of physiological processes, including anxiety. Histaminergic neurons are localized in the tuberomammillary nucleus of the posterior hypothalamus and share bidirectional connections with the lateral septum, an area well implicated in anxiety. The current study examined whether the histaminergic system of the lateral septum regulates rats' defensive behaviors in two animal models of anxiety, the elevated plus maze (EPM) and novelty-induced suppression of feeding paradigm (NISF). We found that bilateral infusions of histamine (1.0 μg and 5.0 μg) into the lateral septum selectively decreased rats' defensive behaviors in the EPM (both doses) and NISF (1.0 μg only). Follow-up studies found that pre-infusions of the H_1 and H_2 antagonists, pyrilamine (20 μg) and ranitidine (20 μg) respectively, reversed the anxiolytic-like effects of intra-LS histamine (1.0 μg) in the NISF but not in the EPM, while pre-infusions of the H_3 antagonist ciproxifan (200 pg) attenuated the anxiolytic-like effects of intra-LS histamine in the EPM but not in the NISF. This double dissociation suggests that H_1 and H_2 receptors in the lateral septum, likely via a post-synaptic mechanism, mediate the anxiolytic-like effects of histamine in the NISF but not in the EPM. In contrast, lateral septal H_3 receptors mediate, likely pre-synaptically, the anxiolytic-like effects of histamine in the EPM but not in the NISF. Our findings indicate that these receptors differentially contribute to rats' specific defensive behaviors in the EPM and NISF, that is, avoidance of open spaces and neophagia respectively.

1. Introduction

Histaminergic neurons are exclusively found in the tuberomammillary nucleus of the posterior hypothalamus (TM), where they project to almost all regions of the brain [1–4]. The neural histaminergic system is implicated in a range of physiological processes including homeostasis, sleep, learning, memory, and more pertinently, anxiety [5]. For example, exposure to stressful conditions increases histamine turnover rates in the rodent brain [6–9], while the opposite occurs following the administration of anxiolytic drugs such as diazepam or buspirone [10,11]. Destruction of the rat TM reduces anxiety-related defensive behaviors in the elevated plus maze, a widely used animal model of anxiety [12]. In this model, rodents generally avoid the open arms and preferentially explore the closed arms; classical benzodiazepine-type anxiolytics reliably increase rats' open-arm exploration [13]. Rats with bilateral TM lesions spend significantly more time in the open arms relative to sham controls [12]. In contrast, open arm exploration is decreased following intraperitoneal (i.p.) injections of L-histidine, a histamine precursor which is converted to histamine in the central nervous system [14].

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Histamine acts via four distinct G-protein coupled receptors: H_1, H_2, H_3, and the recently discovered H_4 [15,16]. H_1 and H_2 receptors are primarily post-synaptic [5,15]. H_3 receptors are exclusively pre-synaptic and can act as autoreceptors [15,17–19] or as heteroreceptors [20–25,15]. While closely related to the H_3 receptor, little is known about the H_4 receptor; indeed, only recently was it even found to be functionally expressed in the brain [26]. Past studies implicate the H_1, H_2, and H_3 receptors in anxiety. For instance, pre-treatment with the H_1 receptor antagonist pyrilamine (i.p.) blocked the anxiogenic-like increase in rats' open-arm avoidance in the EPM following i.p. injections of L-histidine [14]. Similarly, subcutaneous (s.c.) pre-treatment with the H_2 receptor antagonist ranitidine blocked the anxiogenic-like effects of intraperitoneally administered histamine [27]. The H_3 receptor's role in anxiety is more complex. Intraperitoneal injections of either a H_3 receptor agonist (R-α-methylhistamine) or antagonist (thioperamide) did not affect rats' defensive behaviors in the EPM [28]. However, thioperamide (i.p.) decreased immobility in mice in the forced swim test, a model of depression, suggesting that the H_3 receptor may be involved in rodents' depression (but not anxiety)-like behaviors [28]. Similarly, i.p. injections of the selective H_3 agonists R-α-methylhistamine or immepip did not affect rats' anxiety-related behaviors in the EPM or Vogel type conflict test, two classical, benzodiazepine-sensitive models of anxiety in which diazepam produced clear anxiolytic-like effects [29]. In contrast, these same H_3 agonists reduced anxiety-related behaviors in three atypical, antidepressant-sensitive models of anxiety [29]. More specifically, they decreased isolation-induced vocalizations in guinea pig pups, isolation-induced aggressive behavior in the mouse resident–intruder test, and freezing in the rat conditioned fear stress test [29]. Thus, it appears that H_3 receptor activation produces anxiolytic-like effects resembling those of selective serotonin reuptake inhibitors but not those of benzodiazepine anxiolytics [29].

The TM shares bi-directional connections with the lateral septum (LS), an area well known for its role in regulating fear and defensive behaviors [5,30,31]. Exposure to threat or potentially threatening contexts increases neural activity in the LS, as indicated by enhanced *c-fos* expression [31–36]. Accordingly, lesions of the LS reduce anxiety-like behaviors; more specifically, these lesions increase open arm activity in the EPM and decrease burying behavior in the shock probe burying test, another animal model of anxiety [37]. Infusions of midazolam, a benzodiazepine, or muscimol, a $GABA_A$ receptor agonist, into the lateral septum produce similar anxiolytic-like decreases in rats' open arm avoidance and shock probe burying [38,39].

Histamine, when unilaterally infused into the LS, produced anxiogenic-like decreases in open arm exploration in the EPM, and this decrease was attenuated by pre-treatment with either the H_1 antagonist pyrilamine or the H_2 antagonist ranitidine [40]. One limitation of the EPM, however, is that treatment-induced changes in open arm exploration can reflect changes in appetitive motivation rather than changes in anxiety levels [41]. For example, food deprivation increases rats' open arm exploration without affecting their defensive behaviors in non-exploration based models of anxiety [42–44]. Moreover, the LS is known to be involved in reward and motivation [31], and histamine itself is implicated in reinforcement and feeding behaviors [45–47]. Given these potential confounds, it is important to determine whether the previously observed anxiogenic-like effects following intra-LS infusions of histamine [40] reflect changes in anxiety *or* changes in appetitive motivation. One paradigm that can distinguish between the effects of anxiety and those of appetitive motivation is the novelty-induced suppression of feeding (NISF) paradigm. Here, rodents are offered a palatable snack in a familiar (home cage) and an unfamiliar (novel cage) environment [48]. Rodents take markedly longer to begin eating the snack when it is offered in the unfamiliar, novel cage; this neophagic effect is attenuated with anxiolytic drugs [48]. Accordingly, anxiolytic-like effects in this test are indicated by a reduction in the latency to initiate snack consumption in the novel cage without changing response latencies in the home cage. In contrast, treatment-induced changes in appetitive motivation would change the latency to initiate snack consumption in both the home and novel cages. Thus, the initial purpose of the current study was to examine the effect of histamine, when locally infused into the lateral septum, on rats' defensive behaviors in the EPM and NISF tests (Experiment 1). As a follow-up, we wanted to determine if blocking the H_1 (Experiment 2), H_2 (Experiment 3), or H_3 (Experiment 4) receptor would attenuate any histamine-induced effects on behavior. We chose pyrilamine, ranitidine, and ciproxifan as our H_1, H_2, and H_3 antagonists respectively due to their high affinity, selectivity, and efficacy [49–56].

2. Materials and methods

2.1. Subjects

204 experimentally naïve, male Long–Evans rats (Charles River, Quebec) weighing 275–325 g at the time of surgery were used. Upon arrival, the rats were doubly housed in polycarbonate cages (45.5 × 24 × 21 cm) and allowed to acclimatize to the colony for at least 1 week prior to surgery. Following surgery, the rats were singly housed according to standard practice in our lab for surgically prepared animals. The colony was maintained on a regular 12:12 light/dark cycle (lights on at 0700 h) at approximately 21 °C with food and water available ad libitum. All procedures met the regulations established by the Canadian Council on Animal Care and were approved by the Queen's University Animal Care Committee.

2.2. Surgery

The rats were anesthesized with isoflurane (4.5% for induction; 1–2% for maintenance) in oxygen and given buprenorphine (0.015 mg/kg, s.c.) preoperatively to reduce pain. Marcaine (2.0 mg/kg), a local anesthetic, was injected subcutaneously into the incision site; afterwards, the rats were placed into a Kopf stereotaxic instrument. Ten minutes later, the scalp was incised along the midline and burr holes drilled into the skull. Two 23-guage, stainless-steel guide cannulae were bilaterally lowered to 1.5 mm above the lateral septum, according to the following coordinates [57]: from bregma: 0.5 mm AP, ±1.2 mm ML; from dura: −3.4 mm at a 7° medial angle. The guide cannulae were secured to the skull using four small jeweller's screws and dental acrylic. To prevent clogging, a stylet was inserted into each cannula guide after surgery. Immediately following surgery, the rats were injected subcutaneously with the anti-inflammatory analgesic ketoprofen (Anafen; 5 mg/kg) and 5–10 mL Lactated Ringer. Body temperatures were maintained by placing the rats under a heat lamp. After waking from anesthesia, the rats were transferred to a recovery room that was separated from the home colony. For three days post-surgery, the rats received two daily injections of buprenorphine (0.015 mg/kg, s.c.) and a daily injection of Anafen (5 mg/kg, s.c.). The rats were returned to the home colony four days post-surgery.

The surgical procedures for all four experiments were identical. 48 rats underwent surgery in Experiment 1, 53 in Experiment 2, 52 in Experiment 3, and 51 in Experiment 4.

2.3. Drugs and infusions

The drugs used in the current study were as follows: histamine dihydrochloride, the H_1 receptor antagonist pyrilamine maleate, the H_2 antagonist ranitidine hydrochloride, and the H_3 antagonist ciproxifan hydrochloride (Sigma-Aldrich, ON, Canada). All drugs were dissolved in 0.9% saline and stored in aliquots at −20 °C until use. Fresh aliquots were used on each testing day.

For three consecutive days before testing, the rats were habituated to the infusion procedures by moving them to the infusion room, gently restraining them using a towel, and briefly removing and replacing their cannula stylets. On testing days, the rats were restrained, and