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Behavioural phenotype of histamine H_4 receptor knockout mice: Focus on central neuronal functions

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ABSTRACT

The functional expression of H_4 receptors (H_4R) within neurons of the central nervous system has been recently reported, but their role is poorly understood. The present study aims to elucidate the role of neuronal H_4R by providing the first description of the behavioural phenotype of H_4R-deficient (H_4R knockout, H_4R-KO) mice. Mice lacking H_4R underwent behavioural studies to evaluate locomotor activity, pain perception, anxiety, depression, memory and feeding behaviour. H_4R-KO mice showed a significant increase in ambulation in an open field as well as in exploratory activity in the absence of any modification of motor coordination. The sensitivity of mutant mice to a thermal or a mechanical stimulus was identical to that of the wild type mice, but H_4R-KO showed sensory hypersensitivity toward a condition of neuropathic pain. The lack of H_4R is associated with the promotion of anxiety in the light-dark box test. H_4R-KO mice showed an increased immobility time in the tail suspension test, experimental procedure used to evaluate the response of H_4R deficient mice to a behavioural despair paradigm. Cognitive function parameters of H_4R deficient mice, examined using the passive avoidance and the novel object recognition tests, were unaltered showing the lack of influence of H_4R on working and recognition memory. Finally, H_4R-deficient mice showed an orectic phenotype. These results illustrate that H_4R modulates various neurophysiological functions such as locomotor activity, anxiety, nociception and feeding behaviour, confirming the importance of the integrity and functionality of neuronal H_4R in the histaminergic regulation of neuronal functions.

1. Introduction

Histamine activates four G protein-coupled receptors designated H_1R, H_2R, H_3R and H_4R (Bongers et al., 2010; Walter and Stark, 2012; Seifert et al., 2013; Strasser et al., 2013). Peripheral histamine is produced by mast cells, basophils and gastric enterochromaffine-like cells with a key role in immunological processes, allergy and inflammation that involves all histamine receptor subtypes. In addition to its peripheral functions, histamine also acts as a neurotransmitter. The H_1R, H_2R and H_3R as well as the histamine-synthesizing enzyme histidine decarboxylase (HDC) are neuronally expressed (Haas et al., 2008; Schneider et al., 2014a). Neuronal histamine activates postsynaptic H_1R and H_2R and regulates a multitude of behaviours and metabolic functions, e.g. food intake, energy consumption, respiration, susceptibility to seizures, locomotor activity, cognition, pain perception, circadian rhythm, sleep and wakefulness, arousal or emotional states (Schneider et al., 2014a). The release of neuronal histamine is controlled by a negative feedback, which is mediated by the presynaptic H_3R (Haas et al., 2008; Schneider et al., 2014b).

The physiological role of the histamine H_4R, the most recently discovered histamine receptor subtype (Nakamura et al., 2000; Oda et al., 2000; Liu et al., 2001), has not yet been fully elucidated. The immunological function of the peripheral H_4R has been clearly identified (Seifert et al., 2013; Neumann et al., 2014). However, much less is known about expression and function of H_4R in the

Abbreviations: DRG, dorsal root ganglia; H_4R, histamine H_4 receptors; H_4R-KO, Histamine H_4 receptor knockout; i.c.v., intracerebroventricular; i.p., intraperitoneal; NORT, novel object recognition test; SNI, spared nerve injury; TST, tail suspension test; Wt, wild type.

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central nervous system and since the discovery of H_4R, its functional presence in the peripheral and central nervous system has been controversially discussed (Schneider and Seifert, 2016).

The H_4R mRNA was detected in human dorsal root ganglia (DRG), spinal cord and brain regions including hippocampus, cortex, thalamus and amygdala. In the rat H_4R mRNA was found in the DRG, spinal cord, cortex, cerebellum, brainstem, amygdala, thalamus and striatum (Strakhova et al., 2009). In addition, significantly increased H_4R mRNA expression was observed in the putamen and caudate nucleus of Parkinson's patients (Shan et al., 2012). Moreover, on the protein level, H_4R was immunohistochemically identified in the DRG and spinal cord (Strakhova et al., 2009; Lethbridge and Chazot, 2010) as well as in several regions of human and mouse brain, such as thalamus, hippocampus and cerebral cortex (Connelly et al., 2009). The functional expression of H_4R on human and rodent neurons highlights their implication in neuronal functions, but the difficulty to generate H_4R antibodies with high specificity (Beermann et al., 2012) generates results that should be interpreted with some caution.

The use of selective ligands for H_4R can greatly help understand the physiological role of H_4R. However, in the absence of knockout controls, off-target effects of the H_4R ligands cannot be fully excluded. Although H_4R-deficient mice were generated more than a decade ago (Hofstra et al., 2003), they have not yet been used for systematic experiments to elucidate the physiological and pathological role of this receptor subtype within the central nervous system. The aim of the present study is to clarify the functional role of neuronal H_4R and its involvement in neuronal processes through the phenotypic characterization of H_4R-deficient mice.

2. Materials and methods

2.1. Animals and reagents

Histamine H_4 receptor knockout ($H4R^{-/-}$) mice were generated by Lexicon Genetics (Woodlands Park, TX, USA) as previously described (Hofstra et al., 2003) provided by Janssen Research & Development, LLC La Jolla, CA, USA and back crossed to CB57 background. Corresponding wild-type (Wt) mice were obtained from Harlan Laboratories (Bresso, Italy).

Male mice were randomly assigned to standard cages, with four to five animals per cage. The cages were placed in the experimental room 24 h before behavioural test for acclimatization. The animals were fed a standard laboratory diet and tap water ad libitum and kept at 23 ± 1 °C with a 12 h light/dark cycle, light on at 7 a.m. The experimental protocol was carried out after approval by the Animal Care and Research Ethics Committee of the University of Florence, Italy, under license from the Italian Department of Health (54/2014-B) and in compliance with international laws and policies (Directive, 2010/63/EU of the European parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes; Guide for the Care and Use of Laboratory Animals, US National Research Council, 2011). All studies involving animals are reported in accordance with the ARRIVE guidelines for experiments involving animals (McGrath and Lilley, 2015). All effort was taken to minimize the number of animals used and their suffering.

All behavioural experiments were performed during the light phase with a blind procedure. The number of animals per experiment was based on a power analysis (Charan and Kantharia, 2013) and ten animals per group were used to have the probability of 86% that the study detects a difference between groups at a two-sided 0.05 significance level. Sample size was calculated by G power software.

VUF 8430 (40 μg per mouse), diazepam (1 mg/kg) (Sigma, Milan, Italy), D-amphetamine (1 mg/kg; De Angeli, Rome, Italy), amitriptyline (10 mg/kg; Sigma, Milan, Italy) were dissolved in isotonic (NaCl 0.9%) saline solution immediately before use. Drug concentrations were prepared in such a way that the necessary dose could be administered in a volume of 10 ml/kg by intraperitoneal (i.p.) or in a volume of 5 μl per mouse by intracerebroventricular (i.c.v.) injection, as previously described (Galeotti et al., 2003). Doses and administration schedule were chosen on the basis of time-course and dose-response curves performed in our laboratory (Galeotti et al., 2013).

2.2. Locomotor activity

2.2.1. Rotarod test

The apparatus consisted of a base platform and a rotating rod of 3 cm diameter with a non-skid surface. The rod was placed at a height of 15 cm from the base. The rod, 30 cm in length, was divided into 5 equal sections by 6 disks. Thus up to 5 mice were tested simultaneously on the apparatus, with a rod-rotation speed of 16 r.p.m. The integrity of motor coordination was assessed on the basis of the number of falls from the rod in 30 s.

2.2.2. Hole-board test

The spontaneous locomotor activity was evaluated by using the hole-board test. The apparatus consisted of a 40 cm square plane with 16 flush mounted cylindrical holes (3 cm diameter) distributed 4 by 4 in an equidistant, grid-like manner. Mice were placed on the center of the board one by one and allowed to move about freely for a period of 10 min each. Two photobeams, crossing the plane from mid-point to mid-point of opposite sides, thus dividing the plane into 4 equal quadrants, automatically signalled the movement of the animal (counts in 5 min) on the surface of the plane (spontaneous mobility). Miniature photoelectric cells, in each of the 16 holes, recorded (counts in 5 min) the exploration of the holes (exploratory activity) by the mice.

2.3. Nociceptive behaviour

2.3.1. Mechanical threshold (von Frey's test)

Mechanical allodynia was measured by using Dynamic Plantar Aesthesiometer (Ugo Basile, Bologna, Italy). The mice were placed in individual Plexiglas cubicles (8.5 × 3.4 x 3.4 (h) cm) on a wire mesh platform and allowed to acclimate for approximately 1 h, during which exploratory and grooming activity ended. After that, the mechanical stimulus was delivered to the plantar surface of the hind paw of the mouse from below the floor of the test chamber by an automated testing device. A steel rod (2 mm) was pushed with electronic ascending force (0–5 g in 35 s). When the animal withdrew its hind paw, the mechanical stimulus was automatically withdrawn and the force recorded to the nearest 0.1 g. Nociceptive response for mechanical sensitivity was expressed as mechanical paw withdrawal threshold (PWT) in grams. The mean PWT was calculated from six consecutive trials (each performed every 30 min) and averaged for each group of mice.

2.3.2. Hargreaves' plantar test

Thermal nociceptive threshold was measured using Hargreaves' device as described (Hargreaves et al., 1988). Paw withdrawal latency in response to radiant heat (infrared) was assessed using the plantar test apparatus (Ugo Basile, Comerio, Italy). Each mouse was placed under a transparent Plexiglas box (7.0 (d) × 12.5 (w) x 17.0 (h) cm) on a 0.6-cm-thick glass plate and allowed to acclimatize for 1–2 h before recording. The radiant heat source consisted of an infrared bulb (Osram halogen-bellaphot bulb; 8 V, 50 W) that was positioned 0.5 cm under the glass plate directly beneath the hind paw. The time elapsed between switching on the infrared radiant