Neuropharmacology 70 (2013) 131–140

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Selective brain region activation by histamine H_3 receptor antagonist/inverse agonist ABT-239 enhances acetylcholine and histamine release and increases c-Fos expression

L. Munari [1,2], G. Provensi [1], M.B. Passani, P. Blandina*

Dipartimento di Farmacologia Preclinica e Clinica, Universitá degli Studi di Firenze, Viale G. Pieraccini 6, 50139 Firenze, Italy

ARTICLE INFO

Article history:
Received 19 October 2012
Received in revised form
17 January 2013
Accepted 20 January 2013

Keywords:
Acetylcholine
c-Fos
Histamine
Histamine H_3 receptor
Microdialysis

ABSTRACT

Histamine axons originate solely from the tuberomamillary nucleus (TMN) to innervate almost all brain regions. This feature is consistent with a function for histamine over a host of physiological processes, including regulation of appetite, body temperature, cognitive processes, pain perception and sleep–wake cycle. An important question is whether these diverse physiological roles are served by different histamine neuronal subpopulations. Here we report that systemic administration of the non-imidazole histamine H_3 receptor antagonist 4-(2-{2-[(2*R*)-2-methylpyrrolidinyl]ethyl}-benzofuran-5-yl)benzonitrile (ABT-239, 3 mg/kg) increased c-Fos expression dose-dependently in rat cortex and nucleus basalis magnocellularis (NBM) but not in the nucleus accumbens (NAcc) nor striatum, and augmented acetylcholine and histamine release from rat prefrontal cortex. To further understand functional histaminergic pathways in the brain, dual-probe microdialysis was used to pharmacologically block H_3 receptors in the TMN. Perfusion of the TMN with ABT-239 (10 μM) increased histamine release from the TMN, NBM, and cortex, but not from the striatum or NAcc. When administered locally, ABT-239 increased histamine release from the NBM, but not from the NAcc. Systemic as well as intra-TMN administration of ABT-239 increased c-Fos expression in the NBM, and cortex, but not in the striatum or NAcc. Thus, as defined by their sensitivity to ABT-239, histaminergic neurons establish distinct pathways according to their terminal projections, and can differentially modulate neurotransmitter release in a brain region-specific manner. This implies independent functions of subsets of histamine neurons according to their terminal projections, with relevant consequences for the development of specific compounds that affect only subsets of histamine neurones, thus increasing target specificity.

1. Introduction

The histamine H_3 receptor (H_3R) is largely confined to the nervous system (Leurs et al., 2005), where it acts as a presynaptic autoreceptor that restricts histamine release and synthesis (Arrang et al., 1987, 1983). The H_3R is located also on histaminergic somata where it provides a tonic inhibition of firing (Haas and Panula, 2003). However, its presence is not restricted to histaminergic neurons, and H_3 heteroreceptors modulate the release of several neurotransmitters, including acetylcholine (ACh), glutamate, noradrenaline and serotonin, from different brain regions (Blandina et al., 2010). Pharmacological blockade of H_3Rs exert procognitive effects, increase wakefulness and reduce bodyweight in animal models (Passani and Blandina, 2011; Passani et al., 2007). As a result, an increasing number of H_3R antagonists/inverse agonists progressed to clinical trials for the treatment of a variety of conditions, including narcolepsy as well as cognitive and wakefulness disorders associated with Alzheimer's disease, Parkinson's disease, schizophrenia and attention-deficit hyperactivity disorder (ADHD) (Benarroch, 2010; Passani and Blandina, 2011). Moreover, the use of H_3R antagonists/inverse agonists that weaken traumatic memories may alleviate disorders such as post-traumatic stress syndrome, panic attacks, specific phobias and generalized anxiety (Blandina et al., 2010; Passani et al., 2001). Recent evidence suggests more

Abbreviations: H_3R, histamine H_3 receptor; ACh, acetylcholine; ADHD, attention-deficit hyperactivity disorder; i.p., intraperitoneally; PBS, phosphate-buffer saline; TMN, tuberomamillary nucleus; NBM, nucleus basalis magnocellularis; NAcc, Nucleus Accumbens.

* Corresponding author. Tel.: +39 (0)554271239.
E-mail address: patrizio.blandina@unifi.it (P. Blandina).
[1] L. Munari and G. Provensi contributed equally.
[2] Present address: Department of Pharmacology and Systems Therapeutics, Mount Sinai School of Medicine, New York, NY 10029, USA.

0028-3908/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neuropharm.2013.01.021

areas of potential therapeutic interest, such as in the treatment of alcoholism (Nuutinen et al., 2011) and pain (Hsieh et al., 2010). ABT-239 is a selective, non-imidazole H_3R antagonist/inverse agonist with similar high potency in both human and rat (Cowart et al., 2004). In cognition studies, it improved acquisition of a five-trial, inhibitory avoidance test in rat pups, and social memory in adult and aged rats (Fox et al., 2005). In microdialysis studies, it enhanced ACh release from rat frontal cortex and hippocampus as well as dopamine release from the frontal cortex, but not from the striatum (Fox et al., 2005). The goal of the present study was to evaluate whether neurochemical effects induced by ABT-239, such as c-Fos activation, ACh and histamine release modulation, were restricted through region-specific regulation to distinct brain regions involved in cognitive processes.

2. Experimental procedure

2.1. Animals

Male Sprague–Dawley rats (225–275 g) or CD1 mice (25–30 g; both Harlan, Milano, Italy) were used for experiments, all of which complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC) recommendations for the care and use of laboratory animals, and local ethical review. Animals were housed in groups of three/five in a temperature-controlled room (20–24 °C), allowed free access to food and water, and kept on a 12-h light/dark cycle (light starts at 7:00 AM). Alternatives to in vivo techniques were not available, but all efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Microdialysis in freely moving rats

Rats, anesthetized with chloral hydrate (400 mg/kg i.p.) and positioned in a stereotaxic frame (Stellar, Stoelting Co., Wood Dale, IL), were implanted with one or two guide cannulae (Metalant, Sweden) according to the following coordinates from bregma (Paxinos and Watson, 1998): TMN, AP = −4.3, L = −1.1, DV = +7.2; nucleus basalis magnocellularis (NBM), AP = −0.8; L = −2.8; DV = +6.5; Dorsal Striatum, AP = 0, L = −4, DV = +4; Nucleus Accumbens (NAcc), AP = +1,7, L = −1.4, DV = +6.3; Prefrontal Cortex, AP = +3.2; L = −1.0; DV = +2.8. In the experiments aimed at measuring the release of both histamine and ACh from the prefrontal cortex, rats were implanted with two guide cannulae in the prefrontal cortex according to the following coordinates: AP = +3.2; L = +0.8; DV = +1.3 at 12° angle for the ACh probe, and AP = +3.2; L = −0.8; DV = +2.3 at 12° angle for the histamine probe. A surgical screw served as an anchor and the cannulae were fixed to the skull with acrylic dental cement (Fig. 1).

The microdialysis experiments were performed 48 h after surgery during which rats, housed one per cage, recovered from surgery. The stylet was removed from the guide cannulae, and the microdialysis probes were inserted; the probe for ACh (molecular weight cut-off = 20,000 Da, CMA USA) protruded 3 mm from the cannula tip, whereas that for HA (molecular weight cut-off = 6000 Da, Microbiotech, Sweden) protruded 2 mm. Probes were perfused with Ringer's solution (in mM: NaCl, 147; $CaCl_2$, 1.2 and KCl, 4.0 at pH 7.0) at a flow rate of 2 μl/min using a microperfusion

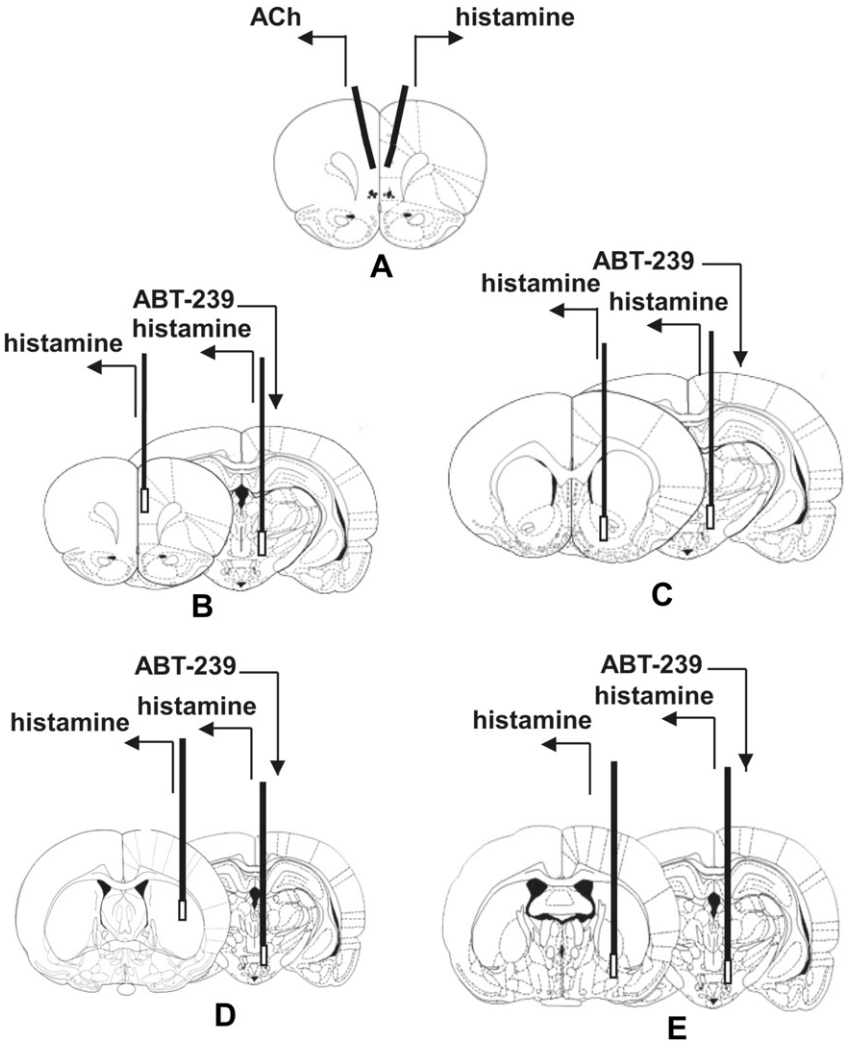

Fig. 1. Schematic diagram showing the position of the microdialysis probes. Rats were implanted with two contralateral probes in the prefrontal cortex to measure the release of ACh and histamine (A). In another set of experiments, rats were implanted with one probe in the TMN to deliver drugs locally and measure neurotransmitter release, and a second probe in the prefrontal cortex (B), the NAcc (C), the dorsal striatum (D) or the NBM (E) to measure histamine release.