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Inflammation Research

2. *Histamine and the central nervous system*

GABAergic mechanism in histamine H_3 receptor inhibition of K^+-evoked release of acetylcholine from rat cortex in vivo

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Introduction

Autoradiographic studies have suggested that the presence of H_3 receptors, initially detected as autoreceptors inhibiting histamine release [1], is not restricted to histaminergic neurons [2–4]. Accordingly, functional studies have shown that H_3 receptors modulate the release of several neurotransmitters, including acetylcholine (ACh) in vitro [5], and in vivo [6]. The present study assessed the location of H_3 receptors modulating ACh release.

Materials and methods

A transversal microdialysis membrane was implanted in both parietal cortices of male Wistar rats (200–250 g), anesthetized with chloral hydrate (400 mg/kg, i.p.). Twenty-four hours after surgery the membrane was perfused with Ringer solution containing 7 μM physostigmine (flow rate: 3 μl/min). Ten minute fractions were collected. ACh and GABA contents were measured by HPLC with electrochemical and fluorometric detection, respectively. Rats were stimulated twice by a 10-min exposure to a 100 mM K^+-medium, given through the dialysis fiber at 50 (S_1) and 140 min (S_2) after equilibration. Spontaneous release values were obtained by averaging ACh and GABA contents in the four samples immediately before S_1. Drugs were added to the medium 10 min before S_2 and maintained during S_2, and their effects evaluated by calculating the ratio of the evoked releases (S_2/S_1). All values are expressed as means ± SEM, with number of experiments (h). All experiments were done in compliance with the recommendations of the EEC (86/609/CEE) for the care and use of laboratory animals and were approved by the Animal Care Committee of the Universitá di Firenze.

Results and discussion

This study demonstrates that H_3 receptors inhibiting cortical ACh release are not presynaptically located, and facilitate the release of GABA from cortical interneurons. Rat cerebral cortex spontaneously released ACh at stable rates, 4.1 ± 0.6 pmol/10 min (n = 33). Two identical 100 mM K^+ stimulations (S_1 and S_2), each more than doubled ACh release (S_2/S_1: 1.33 ± 0.10, n = 6). Tetrodotoxin (0.5 μM), a voltage-dependent Na^+-channel blocker, was infused into the prefrontal cortex 20 min before S_2 and maintained during S_2. K^+-evoked release of ACh failed to show any tetrodotoxin-sensitive component (S_2/S_1: 1.1 ± 0.1, n = 6), thus excluding involvement of neuronal loops. Imetit (10 μM), an H_3 receptor agonist, reduced 100 mM K^+-evoked ACh release in the absence (S_2/S_1: 0.69 ± 0.01, n = 4), but not in the presence of tetrodotoxin (S_2/S_1: 1.1 ± 0.12, n = 4). Hence the suggestion that H_3 receptors modulating ACh release are not located presynaptically on cholinergic nerve terminals, or on non-cholinergic nerve endings impinging on the former. Consistently, H_3 receptor agonists failed to alter [^{3}H]-ACh release from rat cortical synaptosomes [7]. Bicuculline, a $GABA_A$ receptor antagonist, reversed the inhibition of ACh release induced by

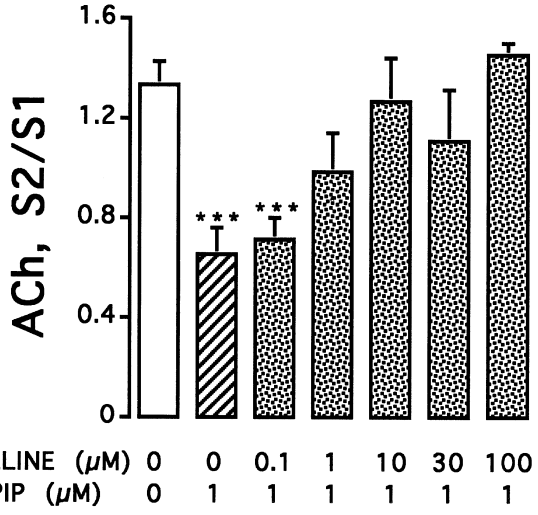

Fig. 1. The influence of bicuculline on immepip-induced inhibition of 100 mM K^+-evoked release of ACh from cortex of freely moving rats. Bicuculline was infused into the prefrontal cortex through the dialysis fiber 20 min before S_2 and maintained during S_2 stimulation. Immepip was infused, alone or along with bicuculline, 10 min before S_2 and maintained during S_2. Shown are the means ± SEM of 3–6 experiments. ***$p < 0.001$ vs control by ANOVA and Scheffe's test.

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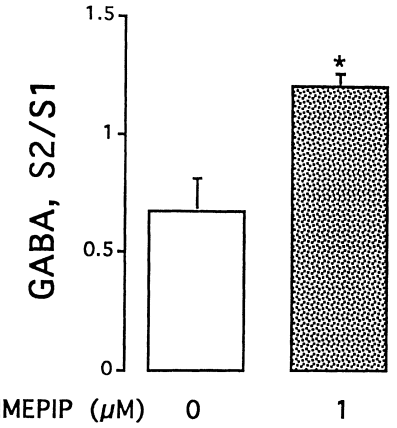

Fig. 2. The influence of immepip on 100 mM K^+-evoked release of GABA from cortex of freely moving rats. Immepip (1 μM) was infused into the prefrontal cortex through the dialysis fiber 10 min before S_2 and maintained during S_2. Rat cerebral cortex spontaneously released GABA at stable rates, 7.9 ± 1.3 pmol/10 min (n = 6). Shown are means ± SEM of 3 experiments. *$p < 0.05$ by unpaired Student's t-test.

immepip, an H_3 receptor agonist (Fig. 1). The addition of 1 μM immepip to the perfusing medium increased 100 mM K^+-evoked release of GABA from the cortex of freely moving rats up to more than 50% (Fig. 2). These findings suggest that H_3 receptors, located postsynaptically on intrinsic perikarya, facilitate the release of GABA, which, in turn, inhibits ACh release.

References

[1] Arrang JM, Garbarg M, Schwartz JC. Auto-inhibition of brain histamine release mediated by a novel class (H_3) of histamine receptor. Nature 1983;302:832–7.
[2] Martinez-Mir MI, Pollard H, Arrang JM, et al. Three histamine receptors (H_1, H_2 and H_3) visualized in the brain of human and non human primates. Brain Res 1990;526:322–7.
[3] Cumming P, Shaw C, Vincent SR. High affinity histamine binding site is the H_3 receptor: characterization and autoradiographic localization in rat brain. Synapse 1991;8:144–51.
[4] Pollard H, Moreau J, Arrang JM, Schwartz J-C. A detailed autoradiographic mapping of histamine H_3 receptors in rat brain areas. Neuroscience 1993;52:169–89.
[5] Clapham J, Kilpatrick GJ. Histamine H_3 receptors modulate the release of [^{3}H]-acetylcholine from slices of rat enthorinal cortex: evidence for the possible existence of H_3 receptor subtypes. Br J Pharmacol 1992:107:919–23.
[6] Blandina P, Giorgetti M, Cecchi M, Leurs R, Timmerman H, Giovannini MG. Histamine H_3 receptor inhibitor of K^+-evoked release of acetylcholine from rat cortex in vivo. Inflamm Res 1996;45:S54–5.
[7] Arrang JM, Drutel G, Schwartz JC. Characterization of histamine H_3 receptors regulating acetylcholine release in rat enthorinal cortex. Br J Pharmacol 1995;114:1518–22.