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Short communication

Sensitivity of histamine H_3 receptor agonist-stimulated [^{35}S]GTPγ[S] binding to pertussis toxin

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Abstract

The effect of histamine H_3 receptor-selective ligands on [^{35}S]guanosine 5'-*o*-(γ-thio)triphosphate ([^{35}S]GTPγ[S]) binding has been examined in rat cerebral cortical membranes. R^{α}-Methylhistamine and N^{α}-methylhistamine produced a concentration-dependent stimulation of [^{35}S]GTPγ[S] binding which was attenuated in the presence of the selective histamine H_3 receptor antagonist thioperamide. In addition, treatment of brain membranes with pertussis toxin abolished the histamine H_3 receptor agonist stimulated binding of [^{35}S]GTPγ[S]. These results provide the first evidence that histamine H_3 receptors couple directly to a G_i/G_o protein in mammalian brain.

Keywords: [^{35}S]GTPγ[S] binding; Histamine H_3 receptor; G-protein; Pertussis toxin

1. Introduction

The ability of pre-synaptic histamine H_3 receptors to inhibit neurotransmitter release in mammalian brain (Hill, 1990) suggests that, like adenosine A_1 receptors (Fredholm et al., 1994), the histamine H_3 receptor can couple to the G_i or G_o class of guanine nucleotide binding proteins (G-proteins). The signal transduction mechanism for histamine H_3 receptors is still unclear. Evidence suggesting that histamine H_3 receptors are coupled to their effector system via a G-protein has come from studies of [^{3}H]H_3 agonist binding, which has been shown to be regulated by guanine nucleotides (Arrang et al., 1990; Zweig et al., 1992; Clark and Hill, 1995). Furthermore, Endou et al. (1993) observed that the histamine H_3 receptor modulation of noradrenaline release from sympathetic nerve endings in guinea pig myocardium was attenuated by pertussis toxin pretreatment, suggesting that histamine H_3 receptors are coupled to a G_i/G_o protein. [^{35}S]GTPγ[S] binding allows measurement of agonist stimulated G-protein activation independently of the second messenger system (Lazareno and Birdsall, 1993). This technique has been used successfully to study several receptor-G-protein interactions in both transfected cells (Lazareno and Birdsall, 1993), and membranes prepared from porcine atria (Hilf et al., 1989), bovine brain (Lorenzen et al., 1993) and rat brain (Sweeney and Dolphin, 1995). The aims of the present study were to investigate histamine H_3 receptor-G-protein coupling in rat cerebral cortical membranes, by observing: (i) the effects of histamine H_3-selective ligands on [^{35}S]GTPγ[S] binding and (ii) the sensitivity of histamine H_3 receptor agonist stimulated binding to pertussis toxin.

2. Materials and methods

2.1. Tissue preparation

Cerebral cortices were dissected from male Hooded Lister rats (300–400 g) and disrupted by hand using a ground glass homogenizer in 20 volumes of Tris · HCl buffer (50 mM, pH 7.4). Homogenates were centrifuged twice at 20 000 × *g* for 10 min. The final pellet was resuspended in 50 volumes of buffer and stored as aliquots at −20°C. Samples were used within one month of preparation and protein concentration was determined by the method of Bradford (1976).

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2.2. [^{35}S]GTPγ[S] binding assay

Membranes (50 μg, pre-treated with adenosine deaminase 1 U/ml), were incubated in 1 ml of assay buffer A (50 mM Tris · HCl, 100 mM NaCl, 10 mM $MgCl_2$, 10 μM GDP, 0.1 nM [^{35}S]GTPγ[S], pH 7.4), for 30 min at 25°C. Non-specific binding was determined in the presence of 10 μM non-radioactive GTPγS. The reaction was terminated by filtration (using a Brandel cell harvester), through Whatman GF/B filters, pre-soaked in ice-cold water. Filters were washed twice with 4 ml ice-cold water and then subjected to liquid scintillation counting (75% efficiency).

2.3. Pertussis toxin pre-treatment

Membranes were incubated in assay buffer B (50 mM Tris · HCl, 1 mM EDTA, 1 mM dithiothreitol, 1 mM $MgCl_2$, 0.1 mM GTP, 1 mM ATP, 10 mM thymidine, 10 mM NAD, 10 mM nicotinamide, pH 7.4), in the presence or absence of pertussis toxin (3 μg/mg protein, pre-activated with dithiothreitol in phosphate buffered saline buffer, 40 mM 10 min), for 60 min at 30°C. Membranes were collected by centrifugation (36000 × g, 15 min, 4°C), then resuspended in assay buffer A, and used immediately for [^{35}S]GTPγ[S] binding.

2.4. Drugs

[^{35}S]GTPγ[S] (specific activity 1200–1400 Ci $mmol^{-1}$) was obtained from NEN DuPont, (Herts., UK). Non-radioactive GTPγS and mepyramine maleate were purchased from Sigma Chemical Co. (Dorset, UK). R^{α}-Methylhistamine dihydrochloride, N^{α}-methylhistamine dihydrochloride and thioperamide were obtained from Smith-Kline French laboratories (Herts., UK) and tiotidine from ICI Pharmaceuticals (Macclesfield, UK).

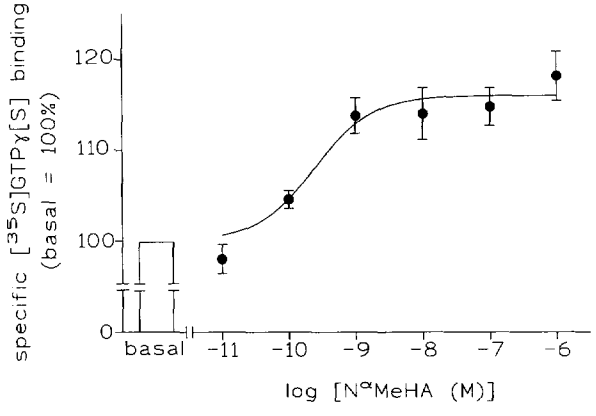

Fig. 1. Stimulation of specific [^{35}S]GTPγ[S] binding by the histamine H_3 receptor agonist, N^{α}-methylhistamine in rat cerebral cortical membranes. Results (mean ± S.E.M.) represent combined data from five separate experiments, in which each point was the mean of six determinations. Data points were fitted to a three-parameter logistic equation using the non-linear regression program Inplot4 (ISI), to the equation: stimulation of specific binding = $(E_{max} - B)/(1 + EC_{50}/A) + B$, where B = basal (i.e. 100%), E_{max} = maximal stimulation, A = agonist concentration and EC_{50} is the concentration of agonist producing half-maximal response. 0.1 μM mepyramine and 10 μM tiotidine were present in all incubations.

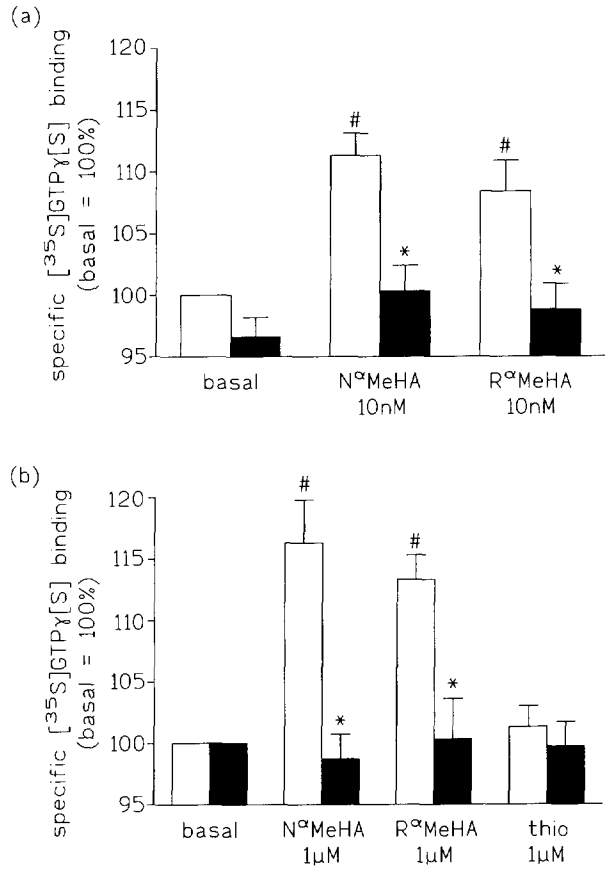

Fig. 2. Effect of (a) thioperamide and (b) pertussis toxin on histamine H_3 agonist stimulated specific [^{35}S]GTPγ[S] binding. Open bars (□) represent control values and closed bars (■) represent specific binding (a) in the presence of 1 μM thioperamide (n = 4), and (b) in membranes pre-treated with pertussis toxin (3 μg/mg protein, n = 3). 0.1 μM mepyramine and 10 μM tiotidine were present in all incubations. $^{\#}P < 0.05$ compared to basal (Student paired t-test) or $^{*}P < 0.05$ compared to control (open bars; paired t-test).

2.5. Data analysis

Data are expressed as mean ± S.E.M. and n represents the number of individual experiments performed. Statistical analysis was performed using a Student paired t-test and $P < 0.05$ was taken as the level for significance.

3. Results

In the presence of selective histamine H_1/H_2 receptor antagonists (mepyramine 0.1 μM and tiotidine

10 μM), the histamine H_3 receptor agonists N^{α}-methylhistamine ($EC_{50} = 0.25 \pm 0.06$ nM, maximal stimulation = $116.0 \pm 1.2\%$; basal = 100%, $n = 5$, $P < 0.01$) (Fig. 1), and R^{α}-methylhistamine ($EC_{50} = 0.42 \pm 0.12$ nM, maximal stimulation = $117.8 \pm 2.5\%$; basal = 100%, $n = 14$, $P < 0.01$), produced a concentration-dependent stimulation of specific [^{35}S]GTPγ[S] binding (specific binding represented 2000–3000 dpm and non-specific binding accounted for 25–35% of total binding). Histamine H_3 receptor agonist stimulated binding was attenuated in the presence of the selective histamine H_3 receptor antagonist, thioperamide (1 μM) (Fig. 2a). Histamine H_3 receptor antagonists were without effect on the non-specific binding of [^{35}S]-GTPγ[S]. Furthermore, specific [^{35}S]GTPγ[S] binding elicited by maximally effective concentrations of histamine H_3 receptor agonists was completely abolished by ADP-ribosylation of the G_{α}-subunit using pertussis toxin (3 μg/mg protein) (Fig. 2b). These data suggest that the response to histamine H_3 receptor agonists is sensitive to inhibition by pertussis toxin across a wide concentration range. The presence of thioperamide or pre-treatment with pertussis toxin had no significant effect on basal [^{35}S]GTPγ[S] binding.

4. Discussion

Our observation that histamine H_3 receptor agonists stimulate specific [^{35}S]GTPγ[S] binding in rat cerebral cortical membranes provides the first direct functional evidence that histamine H_3 receptors are coupled to their effector system via a G-protein. These results substantiate the findings from radioligand binding studies, in which guanylnucleotides have been shown to regulate the binding of radiolabelled histamine H_3 receptor agonists to histamine H_3 receptors (Arrang et al., 1990; Zweig et al., 1992; Clark and Hill, 1995). The inhibition of H_3 receptor-mediated stimulation of [^{35}S]GTPγ[S] binding in brain membranes by pertussis toxin is also consistent with the pertussis toxin sensitivity of the electrophysiological responses to histamine H_3 agonists in guinea-pig myocardium (Endou et al., 1993). These data suggest that the histamine H_3 receptor belongs to the family of G_i/G_o protein coupled receptors.

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