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Involvement of histamine H1 and H2 receptors in the regulation of STAT-1 phosphorylation: Inverse agonism exhibited by the receptor antagonists

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Abstract

Signal transducer and activator of transcription-1 (STAT1) is a latent signal transducer protein which, on phosphorylation, is translocated from the cytoplasm to the nucleus and is subsequently activated. This study was designed to determine the involvement of histamine receptors in histamine-mediated effect on STAT1 phosphorylation. It is known that the actions of histamine mediated through H1 and H2 receptors are dependent on their respective downstream pathways, Ca^{2+}-PKC and cAMP-PKA. In this study, we investigated the significance of PKA in STAT1 phosphorylation. C57BL/6 mouse splenocytes were isolated and treated with histamine (10^{-7}–10^{-4} M) and then activated with PMA (phorbol 12 myristate 13-acetate) plus ionomycin. The phosphorylated STAT1 levels were analyzed by immunoblotting. Histamine receptor agonists amthamine and betahistine, histamine receptor antagonists pyrilamine maleate, tripelennamine, ranitidine, cimetidine and thioperamide, cAMP agonists N^6, 2′-0-dibutyryladenosine-3′,5′-cyclic monophosphate sodium salt (db-cAMP) and forskolin, protein kinase A inhibitors *N*-(2-[*p*-bromocinnamylamino]ethyl)-5-isoquinoline-sulfonamide (H89) and Rp diastereomer of adenosine cyclic 3′,5′-phosphorothioate (RpcAMPs) and tyrosine kinase inhibitor tyrphostin were used to identify the upstream signal transduction pathways. We observed that histamine augmented the phosphorylation of STAT1 through both H1 and H2 receptors. Furthermore, H1 and H2 receptor antagonists displayed inverse agonism. Ca^{2+}-PKC-induced phosphorylation of STAT1 was completely inhibited by H89 and significantly inhibited by RpcAMPs. DbcAMP and

Abbreviations: STAT1, Signal transducer and activator of transcription-1; ICAM-1, Intracellular adhesion molecule-1; PMA, Phorbol 12 myristate 13-acetate; cAMP, Cyclic adenosine monophosphate; db-cAMP, N^6, 2′-0-dibutyryladenosine-3′,5′-cyclic monophosphate sodium salt; PKA, Protein kinase A; H89, *N*-(2-[*p*-bromocinnamylamino]ethyl)-5-isoquinoline-sulfonamide; RpcAMPs, Rp diastereomer of adenosine cyclic 3′,5′-phosphorothioate; PKC, Protein kinase C.

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forskolin augmented the Ca^{2+}-PKC-induced STAT1 phosphorylation thus suggesting a convergent crosstalk between the two histamine receptor signaling pathways, PKA and PKC.

Keywords: Asthma; STAT1; Histamine; Inverse agonism; PKA; Crosstalk

1. Introduction

Histamine is a key mediator of the airway inflammatory events including goblet cell hypersecretion [1], airway hyperresponsiveness [2], eosinophilia [2] and smooth muscle hyperplasia [3]. The immunoregulatory role of histamine in asthma has been well established, primarily through the histamine–cytokine network [4–8]. We have previously demonstrated that histamine augmented the phosphorylation of STAT1 [9], a signal transducer protein mediating the inflammatory cell recruitment to the airways through the gene expression of adhesion molecule ICAM-1 and chemokine RANTES [10–13]. Signal transducer and activator of transcription-1 (STAT1) is latent in cytoplasm and is activated by phosphorylation at the tyrosine residue [14]. Several ligands phosphorylate and activate STAT1 through their receptor-associated and other downstream kinases.

Protein kinase C (PKC) and protein kinase A (PKA) mediate most of the immunomodulatory effects of histamine. Studies have suggested that PKC plays an essential role in the phosphorylation of STAT1. However, the role of PKA in the phosphorylation process has not yet been determined.

In this study we investigated the involvement of H1 and H2 receptors in the histamine-mediated effect on the phosphorylation of STAT1 in mouse splenocytes. This study further suggests a significant role of PKA in this process. Factors regulating STAT1 phosphorylation reveal pathways that mediate the histamine-induced proinflammatory events. STAT-1 pathway is important in the inflammatory process of asthma.

2. Materials and methods

2.1. Materials

Eight to ten week old female C57BL/6 mice were obtained from Charles River (Wilmington, MA). The culture medium RPMI 1640, Hank's balanced salt solution (HBSS), HEPES buffer, sodium pyruvate, sodium bicarbonate, fetal bovine serum (FBS), 2-mercaptoethanol (2-ME), L-glutamine, penicillin/streptomycin, amphotericin and glucose were all purchased from Sigma Aldrich Co. (Saint Louis, MO). Phorbol-12-myristate 13-acetate (PMA) (Sigma Aldrich) plus ionomycin (Sigma Aldrich Co.) were used to activate the C57BL/6 female mouse splenocytes. Histamine dihydrochloride, betahistine, pyrilamine maleate, ranitidine hydrochloride, cimetidine, thioperamide, N^6, 2′-0-Dibutyryladenosine-3′,5′-cyclic monophosphate sodium salt (db-cAMP), *N*-(2-[*p*-Bromocinnamylamino]ethyl)-5-isoquinoline-sulfonamide (H89), Rp-diastereomer of adenosine cyclic 3′,5′-phosphorothioate (Rp-cAMPs triethylamine) and tyrphostin were purchased from Sigma Aldrich Co. Amthamine was purchased from Biomol (Plymouth meeting, PA). The activated splenocytes were lysed with Triton X-100 detergent, purchased from Biorad (Richmond, CA) and with aprotinin bovine lung, pepstatin A, leupeptin hemisulfate salt, sodium pyrophosphate, sodium orthovanadate, sodium fluoride, phenylmethylsulfonylfluoride, Trizma® base and ethylenediaminetetraacetic acid, purchased from Sigma Aldrich Co. Protein standard for the protein assay, gamma globulin, was purchased from Biorad (Richmond, CA). Acrylamide, sodium dodecyl sulphate, ammonium persulphate, *N*,*N*,*N*′,*N*′-Tetramethylethylenediamine (TEMED) and glycine used for SDS-PAGE and electrophoretic transfer were purchased from Sigma Aldrich Co. For the detection of the phospho-STAT1 and STAT1 levels, phospho-Stat1 (Tyr701) antibody, STAT1 antibody, anti-rabbit IgG HRP-linked antibody, anti-biotin HRP-linked antibody, biotinylated protein ladder, LumiGLO® reagent and peroxide solution were all purchased from Cell Signaling Technology (Beverly, MA). XAR films were purchased from Sigma Aldrich Co.