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The 5-HT3 receptor antagonist tropisetron inhibits T cell activation by targeting the calcineurin pathway

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Abstract

Tropisetron, an antagonist of serotonin type 3 receptor, has been investigated in chronic inflammatory joint process. Since T cells play a key role in the onset of several inflammatory diseases, we have evaluated the immunosuppressive activity of tropisetron in human T cells, discovering that this compound is a potent inhibitor of early and late events in TCR-mediated T cell activation. Moreover, we found that tropisetron specifically inhibited both IL-2 gene transcription and IL-2 synthesis in stimulated T cells. To further characterize the inhibitory mechanisms of tropisetron at the transcriptional level, we examined the DNA binding and transcriptional activities of NF-κB, NFAT and AP-1 transcription factors in Jurkat T cells. We found that tropisetron inhibited both the binding to DNA and the transcriptional activity of NFAT and AP-1. We also observed that tropisetron is a potent inhibitor of PMA plus ionomycin-induced NF-κB activation but in contrast TNFα-mediated NF-κB activation was not affected by this antagonist. Finally, overexpression of a constitutively active form of calcineurin indicated that this phosphatase may represent one of the main targets for the inhibitory activity of tropisetron. These findings provide new mechanistic insights into the anti-inflammatory activities of tropisetron, which are probably independent of serotonin receptor signalling and highlight their potential to design novel therapeutic strategies to manage inflammatory diseases.

Keywords: Tropisetron; T cells; IL-2; NF-κB; NFAT; Calcineurin

1. Introduction

5-Hydroxytryptamine (5-HT[1], serotonin) is a well-characterized neurotransmitter that plays a crucial role in the regulation of central processes, such as mode, appetite, sleep and other body rhythms. Moreover, 5-HT is found in the immune-inflammatory axis and has been shown to influence the immune response in mammals [1,2]. The pleiotropic activity of 5-HT is due to the molecular complexity of 5-HT receptors (5-HTR) and their wide tissue expression [3]. Multiple serotoninergic receptors have been identified so far and among them the subtype 5-HT_3 receptor (5-HT_3R), which is an ionotropic receptor permeant to cations with high selectivity to Na^+ inward movements, has been found to be expressed in cells of the immune system including T lymphocytes [4,5], and evidence exists that 5-HT can modulate the T cells functionality through activation of 5-HT_3R [4,6]. Interestingly, highly selective 5-HT_3R antagonists, such as tropisetron, have been investigated in chronic inflammatory joint process, although the antiphlogistic mechanisms of action are largely unknown [7,8].

The signal transduction pathways triggered by the activation of the TCR–CD3 complex in T cells lead to the

Abbreviations: AP-1, activator protein-1; EMSA, electrophoretic mobility shift assay; 5-HT, serotonin; 5-HTR, serotonin receptor; IKK, IκB kinase; IκB, κB inhibitor; JNK, jun kinase; MAPK, mitogen activated kinase; NF-κB, nuclear factor kappa B; NFAT, nuclear factor of activated cells; TNFα, tumor necrosis factor α

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immediate activation of transcription factors that regulate a variety of activation-associated genes. Many of them are cytokines and surface receptors that play an important role in coordinating the immune response [9]. The signal transduction pathways involved in T cell activation are initiated by the clustering of lipids rafts at the cell surface, with formation of a supramolecular activation complex localized at the antigen-induced immunological synapse [10]. Several studies have demonstrated that the presence of specific signalling proteins such as Cot/Tpl-2, Vav-1, PKCθ and PLCγ1 within lipids rafts control lymphocyte signalling [11,12]. Activated PLCγ1 cleaves phosphatidylinositol 4,5 bisphosphate yielding inositol (1,4,5) triphosphate (IP_3) and diacylglycerol (DAG). While IP_3 mobilizes Ca^{2+} from intracellular stores, DAG mediates activation of the protein kinase C (PKC) family members [13]. As a consequence of an increase in intracellular Ca^{2+} levels, several signalling pathways are activated in T cells [14]. In this sense, calcineurin, a Ca^{2+}-calmodulin dependent protein phosphatase, is activated and subsequently dephosphorylates the nuclear factor of activated T cells (NFAT), allowing its nuclear shuttling [14]. This transcription factor was first described as an inducible regulatory complex critical for transcriptional induction of IL-2 in activated T cells, but was subsequently shown to regulate the transcription of many other genes, including cytokines, cell surface receptors and regulatory enzymes [14,15]. In the nucleus, NFAT binds to the DNA either alone or in conjunction with AP-1 proteins [16]. Nevertheless, the coordinated induction and activation of the transcription factors NFAT, NF-κB and AP-1 is required to regulate cytokine gene expression [17].

Stimulation via TCR–CD3 complex alone is sufficient for NFAT activation, but it is insufficient for activation of NF-κB and AP-1. Thus, a second signal mediated by the CD28 co-stimulary receptor is required for the induction of NF-κB and AP-1 in antigen stimulated T cells [18]. The transcription factor NF-κB is one of the key gene regulators involved in the immune/inflammatory response as well as in survival from apoptosis [18]. NF-κB is an inducible transcription factor made up of homo- and heterodimers of p50, p65 (RelA), p52, RelB and c-rel subunits that interact with a family of inhibitory IκB proteins, of which IκBα is the best characterized [19,20]. In most cell types, these proteins sequester NF-κB in the cytoplasm by masking its nuclear localization sequence, and in response to a variety of stimuli, including TCR and CD28 co-stimulation, IκBs are phosphorylated by the IκB kinase complex, followed by their ubiquitination and degradation in the proteasome. The release of IκBs unmasks the NLS and allows NF-κB to enter the nucleus [18,21].

In this paper, we studied the effect of tropisetron, a 5-HT_3R antagonist, on early and late T cell activation events and we have demonstrated that tropisetron inhibits antigen-induced proliferation and IL-2 production in human peripheral T cells. Moreover, we show here for the first time that tropisetron inhibits the signalling pathways that regulate the activation of the transcription factors NFAT, NF-κB and AP-1, which are known to play a critical role in the immune response.

2. Material and methods

2.1. Cell lines and reagents

The 5.1 clone (obtained from Dr. N. Isräel, Institut Pasteur, Paris, France) line is a Jurkat derived clone stably transfected with a plasmid containing the luciferase gene driven by the HIV-LTR promoter and was maintained in exponential growth in RPMI 1640 (Gibco BRL–Life technologies, Barcelona, Spain) supplemented with 10% heat inactivated foetal calf serum, 2 mM L-glutamine, 1 mM Hepes and antibiotics (completed medium) (Gibco BRL–Life technologies, Barcelona, Spain) and G418 (200 μg/ml). Jurkat cells (ATCC, Rockville, MD, USA) were also maintained in exponential growth in complete medium. The anti-IκBα mAb 10B was a gift from R.T. Hay (St. Andrews, Scotland), the mAb anti-tubulin was purchased from Sigma Co (St. Louis, MO, USA), the rabbit polyclonal and anti-p65 (1226) was a gift from A. Isräel (Institute Pasteur, Paris, France). The anti-phospho-ERK 1 + 2 (sc-7383) was from Santa Cruz Biotechnology (CA, USA), the mAbs anti-phospho-p38 (9211S), anti-phospho-JNK (9255S) and anti-phospho-p65 (3031S) were from New England Biolabs (Hitchin, UK). [γ-^{32}P]ATP (3000 Ci/mmol) was purchased from Perkin-Elmer (Boston, MA, USA). All other reagents were from Sigma.

2.2. Plasmids

The AP-1-Luc plasmid was constructed by inserting three copies of an SV40 AP-1 binding site into the Xho site of pGL-2 promoter vector (Promega, MA, USA), the NFAT-Luc plasmid contains three copies of the NFAT binding site of the IL-2 promoter fused to the luciferase gene [22]. The KBF-Luc contains three copies of the MHC enhancer κB site upstream of the conalbumin promoter followed by the luciferase gene [23]. The IL-2-Luc (−326 to +45 of the IL-2 promoter) was previously described [22]. The plasmid pEF-BOS trunk-Cot containing the truncated active form of the Cot kinase was obtained from S. Alemany (CSIC, Madrid, Spain). The expression plasmid ΔCaM-AI encodes a truncated form of a murine calcineurin catalytic subunit that has Ca^{2+} independent and constitutive phosphatase [24]. The Gal4-Luc reporter plasmid includes five Gal4 DNA-binding sites fused to the luciferase gene. The Gal4-hNFAT1 contains the first 1–415 amino acids of human NFAT1 fused to the DNA binding domain of yeast Gal4 transcription factor and was previously described [25]. The Gal4-p65 contains the C-terminal region of the human p65 (amino acids 286–551)