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Diverse signalling by different chemokines through the chemokine receptor CCR5

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Abbreviations:
CHO cells, Chinese hamster ovary cells
ECL, enhanced chemiluminescence
FCS, foetal calf serum
FITC, fluorescein isothiocyanate
GPCR, G protein-coupled receptor
HIV, human immunodeficiency virus
MCP, monocyte chemoattractant protein
MIP, macrophage inflammatory protein
PBS, phosphate-buffered saline
RANTES, regulated on activation, normal T cell expressed and secreted

ABSTRACT

We have investigated the signalling properties of the chemokine receptor, CCR5, using several assays for agonism: stimulation of changes in intracellular Ca^{2+} or CCR5 internalisation in CHO cells expressing CCR5 or stimulation of [^{35}S]GTPγS binding in membranes of CHO cells expressing CCR5.

Four isoforms of the chemokine CCL3 with different amino termini (CCL3, CCL3(2–70), CCL3(5–70), CCL3L1) were tested in these assays in order to probe structure/activity relationships. Each isoform exhibited agonism. The pattern of agonism (potency, maximal effect) was different in the three assays, although the rank order was the same with CCL3L1 being the most potent and efficacious. The data show that the amino terminus of the chemokine is important for signalling. A proline at position 2 (CCL3L1) provides for high potency and efficacy but the isoform with a serine at position 2 (CCL3(2–70)) is as efficacious in some assays showing that the proline is not the only determinant of high efficacy.

We also increased the sensitivity of CCR5 signalling by treating cells with sodium butyrate, thus increasing the receptor/G protein ratio. This allowed the detection of a change in intracellular Ca^{2+} after treatment with CCL7 and Met-RANTES showing that these ligands possess measurable but low efficacy.

This study therefore shows that sodium butyrate treatment increases the sensitivity of signalling assays and enables the detection of efficacy in ligands previously considered as antagonists. The use of different assay systems, therefore, provides different estimates of efficacy for some ligands at this receptor.

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1. Introduction

The G protein-coupled chemokine receptor, CCR5, was originally characterised as a receptor responding functionally to the CC-chemokines CCL3 (MIP-1α), CCL4 (MIP-1β) and CCL5 (RANTES) [1]. CCR5 was subsequently described as the primary co-receptor for macrophage tropic human immunodeficiency virus type 1 (HIV-1) [2–5].

Chemokine interaction with CCR5 initiates several events. Receptor associates with G proteins, leading to activation of signalling processes, e.g. changes in Ca^{2+} followed by receptor phosphorylation by G protein-coupled receptor kinases (GRKs) [6]. This results in association of β-arrestin with the receptor [7,8] and desensitisation via uncoupling of receptor and G protein. A number of CC-chemokines (CCL3, CCL4, CCL5, CCL7, CCL8 and CCL13) bind to CCR5 with different affinities and abilities to activate the receptor [9]. These chemokines can be divided in two subgroups based on amino acid sequence identity [10,11]. CCL3, CCL4 and CCL5 form one subgroup and are full agonists, whereas CCL7, CCL8 and CCL13 form a second subgroup, which share ~60% amino acid identity within the group and ~30% identity with CCL3, CCL4 and CCL5. CCL7 has been reported to bind to CCR5 but cannot activate the receptor in a number of tests [8,9]. CCL8 and CCL13 are partial agonists in some tests, with CCL13 exhibiting a reduced potency. Chemically modified chemokines have been used to study ligand–receptor interaction further. AOP-RANTES [12,13] and AOP-MIP-1α [14] are very potent ligands in several assays. Met-RANTES was described as an antagonist of several signalling processes linked to CCR5 [15]. Subsequently, Met-RANTES was shown to have weak activity in eliciting a Ca^{2+} response in Chinese hamster ovary cells (CHO cells) expressing CCR1, CCR3, and CCR5 [16] but no intrinsic activity on Ca^{2+} transients in eosinophils [17].

Two non-allelic isoforms of CCL3 containing 70 amino acids have been described and termed CCL3 and CCL3L1 (Fig. 1) [18]. These isoforms exhibit >90% homology, differing by only three amino acids. Both forms are transcribed and secreted from mammalian cells [19–22]. The mature form of CCL3 (CCL3(5–70)) lacks the four N-terminal amino acids [21,22]. CCL3L1 has been reported to be much more potent for binding to and activating CCR5 than the naturally occurring CCL3 variants [18,23]. These differences have been attributed to the change of the serine to a proline at position 2. Another CCL3 variant has been tested where the Ala at position 1 is lacking (CCL3(2–70)). This CCL3 variant has been used to create a mutant chemokine, where a D27A mutation reduces self-association to form high-molecular mass aggregates [24]. These CCL3 isoforms (CCL3, CCL3L1, CCL3(5–70), CCL3(2–70), CCL3(2–70)D27A), therefore, provide an interesting group of molecules to probe the properties of CCR5 and the relation of structure and function for the chemokine.

As outlined above, some chemokines, e.g. Met-RANTES, CCL13 exhibit low efficacy in some systems but no efficacy in others. It is important to define the relative efficacy of such compounds and this may require increasing the sensitivity of currently used assay systems. Sodium butyrate has been shown to activate viral promoters and enhance protein expression in stably transfected cells, when a viral promoter is used to express the protein [25]. We showed that sodium butyrate increases cell surface CCR5 in stably transfected CHO cells [8]. Here we used sodium butyrate treatment of cells to increase the number of receptors and enhance the activation of CCR5. The data show that sodium butyrate treatment is a useful tool for investigating signalling events induced by low efficacy agonists.

In the present study, therefore, we employed three assays for agonists at CCR5: stimulation of changes in intracellular Ca^{2+}, stimulation of [^{35}S]GTPγS-binding and receptor internalisation assays [8]. These assays were used to examine the CCL3 isoforms mentioned above. Also a range of chemokines including some low efficacy chemokines (Met-RANTES, CCL13) were examined for their activity in membranes of CHO cells expressing CCR5 treated with sodium butyrate.

2. Materials and methods

2.1. Cells and materials

CHO cells stably expressing CCR5 (CHO·CCR5) or CCR5 and CD4 (CHO·CCR5·CD4) were grown as described previously [7,8]. For treatment with sodium butyrate, cells were incubated for 18 h with 5 mM or 10 mM sodium butyrate prior to assaying.

CCL3, CCL4, CCL5, CCL7, CCL8, CCL13 and MetRANTES were purchased from PeproTech (Rocky Hill, NJ), CCL3(5–70) was from R&D Systems (Abingdon Oxon, UK), the CCL3(2–70) and CCL3(2–70)D27A chemokines were a generous gift from British Biotech (Oxford, UK) and described previously [24]. Secondary antibodies were obtained from Sigma (Poole, UK). Anti-CCR5 antibodies HEK/1/85a/7a have been described previously [7,8], anti-$G\alpha i_{1-3}$ protein antibody was obtained from Santa Cruz (Holy Ditch Farm, UK). All other chemicals were from Sigma (Poole, UK).

2.2. Internalisation assay and flow cytometry analysis

CHO·CCR5 and CHO·CCR5·CD4 cells were incubated with serum-free medium for 2 h at 37 °C, harvested with 2 mM

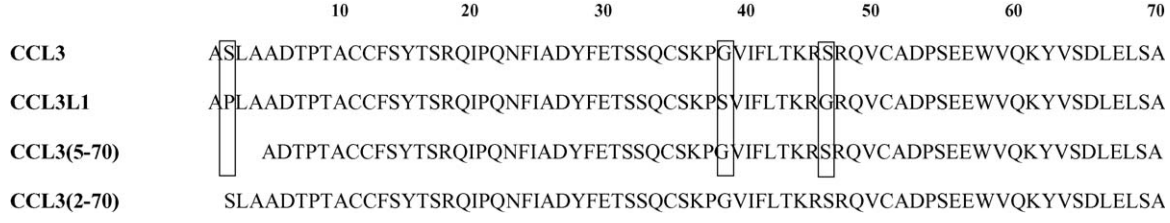

Fig. 1 – Amino acid sequences of the four CCL3 isoforms used. The positions of amino acid residues (2, 39 and 47) where changes occur in the different isoforms are boxed.