Drug Binding Poses Relate Structure with Efficacy in the μ Opioid Receptor

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Abstract

The μ-opioid receptor (MOPr) is a clinically important G protein-coupled receptor that couples to $G_{i/o}$ proteins and arrestins. At present, the receptor conformational changes that occur following agonist binding and activation are poorly understood. This study has employed molecular dynamics simulations to investigate the binding mode and receptor conformational changes induced by structurally similar opioid ligands of widely differing intrinsic agonist efficacy, norbuprenorphine, buprenorphine, and diprenorphine. Bioluminescence resonance energy transfer assays for G_i activation and arrestin-3 recruitment in human embryonic kidney 293 cells confirmed that norbuprenorphine is a high efficacy agonist, buprenorphine a low efficacy agonist, and diprenorphine an antagonist at the MOPr. Molecular dynamics simulations revealed that these ligands adopt distinct binding poses and engage different subsets of residues, despite sharing a common morphinan scaffold. Notably, norbuprenorphine interacted with sodium ion-coordinating residues $W293^{6.48}$ and $N150^{3.35}$, whilst buprenorphine and diprenorphine did not. Principal component analysis of the movements of the receptor transmembrane domains showed that the buprenorphine-bound receptor occupied a distinct set of conformations to the norbuprenorphine-bound receptor. Addition of an allosteric sodium ion caused the receptor and ligand to adopt an inactive conformation. The differences in ligand–residue interactions and receptor conformations observed here may underlie the differing efficacies for cellular signalling outputs for these ligands.

Introduction

The μ-opioid receptor (MOPr) is a $G_{i/o}$ coupled receptor from the class A G protein-coupled receptor (GPCR) family. It is responsible for both the analgesic and euphoric effects of many opioid drugs [1] and is therefore a protein of very significant clinical and societal importance.

The process of GPCR activation, and particularly the molecular difference between high and low efficacy agonists, is poorly understood. The current consensus is that ligand binding induces changes in residue orientation around the ligand binding pocket, termed micro-switches, that translate to larger rearrangements of the intracellular regions of the receptor, hence promoting engagement with intracellular signalling partners such as G proteins and arrestins [2,3]. One well-established hallmark of receptor activation is the outward movement of the lower part of transmembrane domain (TM) 6 and the concurrent small inward shifts of TM5 and TM7, thus opening an intracellular cavity in the receptor into which G protein or arrestin can bind [4–9].

Residues forming a conserved network of polar interactions allosterically connecting the ligand binding site and the intracellular face of MOPr [9–11] also include those that comprise an allosteric sodium ion binding site [12]. Sodium has been previously described as a negative allosteric modulator of MOPr and other class A GPCRs [13–18], and a high-resolution X-ray crystal structure of the δ-opioid receptor bound to an antagonist revealed a sodium

ion coordinated by conserved residues below the ligand binding pocket [19]. These residues have been proposed to be involved in signal transmission from the ligand binding pocket to the G protein binding site [11,20–23]. However, there is limited understanding of the precise nature of this signal transmission through the protein and hence the molecular nature of agonist efficacy.

The MOPr ligands norbuprenorphine, buprenorphine, and diprenorphine share the same morphinan scaffold (Fig. 1a), and all exhibit affinities for MOPr in the nanomolar range [24,25] yet display fundamental differences in intrinsic efficacy. Norbuprenorphine, a metabolite of buprenorphine [26], is a full agonist at MOPr, able to activate G proteins and recruit arrestin-3, whilst buprenorphine is a MOPr partial agonist, producing a submaximal activation of G protein, and is unable to induce measurable arrestin-3 recruitment to the receptor [27]. Diprenorphine is a MOPr antagonist [24], that is, it has extremely low or zero efficacy. In this study, we first confirmed the signalling characteristics of these ligands using bioluminescence resonance energy transfer (BRET) assays. Then, these structurally related ligands were used in molecular dynamics (MD) simulations of MOPr to explore ligand binding poses, residue interactions, and MOPr conformations, which may confer the different abilities of these ligands to engage intracellular signalling partners.

Results

Agonist-induced G protein activation and arrestin-3 recruitment

Human embryonic kidney 293 (HEK 293) cells expressing HA-tagged rat MOPr, Gαi-renilla luciferase (Rluc) II, and Gβγ-green fluorescent protein (GFP) were used to detect dissociation of the Gα and Gβγ subunits upon activation. A decrease in the BRET ratio compared to cells treated with media or 0.01% DMSO alone indicated dissociation, or rearrangement, of these subunits [28]. HEK 293 cells expressing rat MOPr-yellow fluorescent protein (YFP) and arrestin-3-Rluc were used to detect ligand-induced recruitment of arrestin-3 to MOPr. An increase in this BRET ratio indicates arrestin-3 and MOPr coming into closer proximity [29]. Concentration-response curves for G_i activation and arrestin-3 recruitment by the MOPr ligands compared to the standard full agonist DAMGO

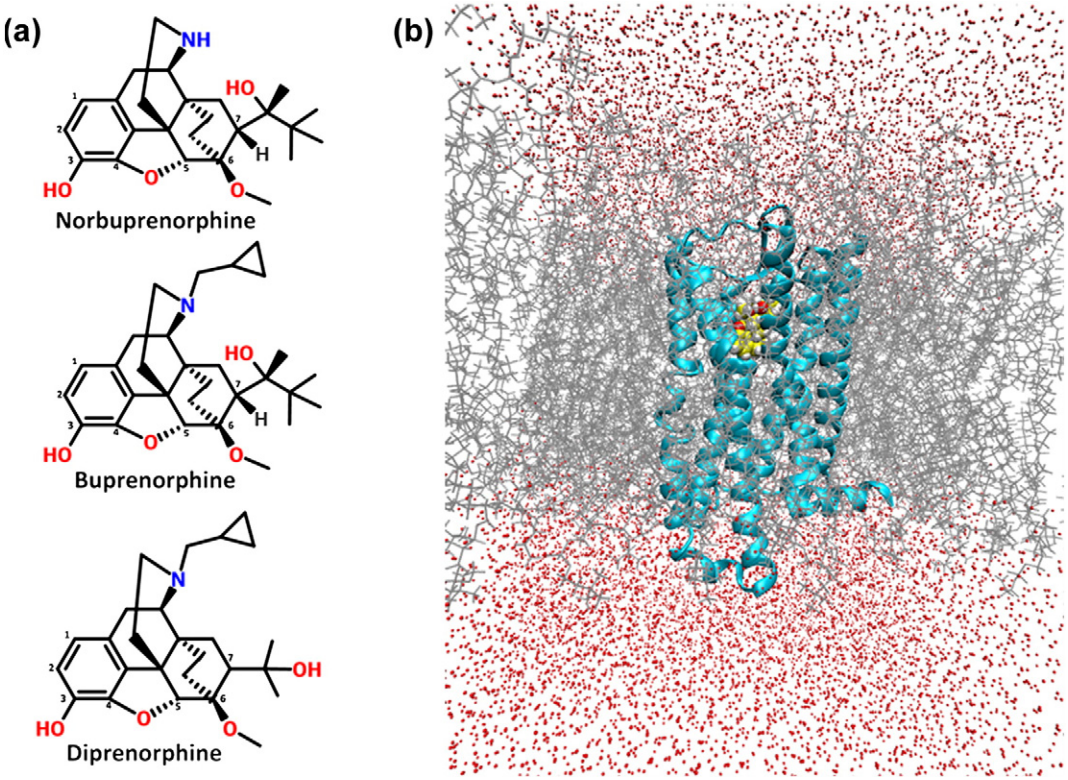

Fig. 1. Structurally similar ligands were used in MD simulations bound to MOPr. (a) Structures of the MOPr ligands used in this study, sharing the same morphinan scaffold. Carbons 1–7 are labelled. (b) Model of MOPr (cyan) bound to a ligand, norbuprenorphine (yellow), and embedded in a cholesterol and phospholipid bilayer (grey) solvated in water and NaCl (red), developed from the antagonist-bound crystal structure [31].