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Distinct pharmacological properties of morphine metabolites at G_i-protein and β-arrestin signaling pathways activated by the human μ-opioid receptor

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ABSTRACT

Morphine and several other opioids are important drugs for the treatment of acute and chronic pain. Opioid-induced analgesia is predominantly mediated by the μ-opioid receptor (MOR). When administered to humans, complex metabolic pathways lead to generation of many metabolites, nine of which may be considered major metabolites. While the properties of the two main compounds, morphine-6-glucuronide and morphine-3-glucuronide, are well described, the activity of other morphine metabolites is largely unknown. Here we performed an extensive pharmacological characterization by comparing efficacies and potencies of morphine and its nine major metabolites for the two main signaling pathways engaged by the human MOR, which occur via G_i-protein activation and β-arrestins, respectively. We used radioligand binding studies and FRET-based methods to monitor MOR-mediated G_i-protein activation and β-arrestin recruitment in single intact 293T cells. This approach identified two major groups of morphine metabolites, which we classified into "strong" and "weak" receptor ligands. Strong partial agonists morphine, morphine-6-glucuronide, normorphine, morphine-6-sulfate, 6-acetylmorphine and 3-acetylmorphine showed efficacies in the nanomolar range, while the weak metabolites morphine-N-oxide, morphine-3-sulfate, morphine-3-glucuronide and pseudomorphine activated MOR pathways only in the micromolar range. Interestingly, three metabolites, normorphine, 6-acetylmorphine and morphine-6-glucuronide, had lower potencies for Gi-protein activation but higher potencies and efficacies for β-arrestin recruitment than morphine itself, suggesting that they are biased towards β-arrestin pathways.

1. Introduction

Morphine is an important alkaloid from opium poppy and serves as the prototypical analgesic drug. Morphine and some other opioids, for example 3,6-di-acetylmorphine (heroin) are also relevant for drug abuse. Analgesic effects of opioids are achieved largely via activation of μ-opioid receptors (MOR) [1,2]. MOR is a G-protein-coupled receptor (GPCR), which can activate several cellular signaling pathways. The first pathway is mediated by the activation of inhibitory pertussis-toxin sensitive G-proteins ($G_{i/o}$-proteins). The second pathway is induced by the recruitment of β-arrestins to the receptor, which leads to subsequent activation of other, so-called "non-classical" signaling cascades such as the mitogen activated protein kinase pathway [3,4].

When administered to humans, morphine and other opiate alkaloids undergo extensive biotransformation by various metabolic pathways. The predominant metabolic pathway involves conjugation with glucuronic acid, which leads to the major metabolites morphine-3-glucuronide and morphine-6-glucuronide [5]. Other metabolic pathways include conjugation with sulfonic acid, leading to morphine-6-sulfates and morphine-3-sulfates, acetylation to 3- and 6-acetylmorphines, oxidation to morphine-N-oxide and demethylation to normorphine [6,7] (Fig. 1). Among all known morphine metabolites, the pharmacological properties have been best studied for morphine-6-glucuronide and morphine-3-glucuronide, which are generally considered as active and inactive metabolites, respectively [8].

Abbreviations: DAMGO, [D-Ala^2, N-MePhe^4, Gly-ol]-enkephalin; FRET, fluorescence (or: Förster) resonance energy transfer; GPCR, G-protein-coupled receptor; GRK, G-protein-coupled-receptor kinase.

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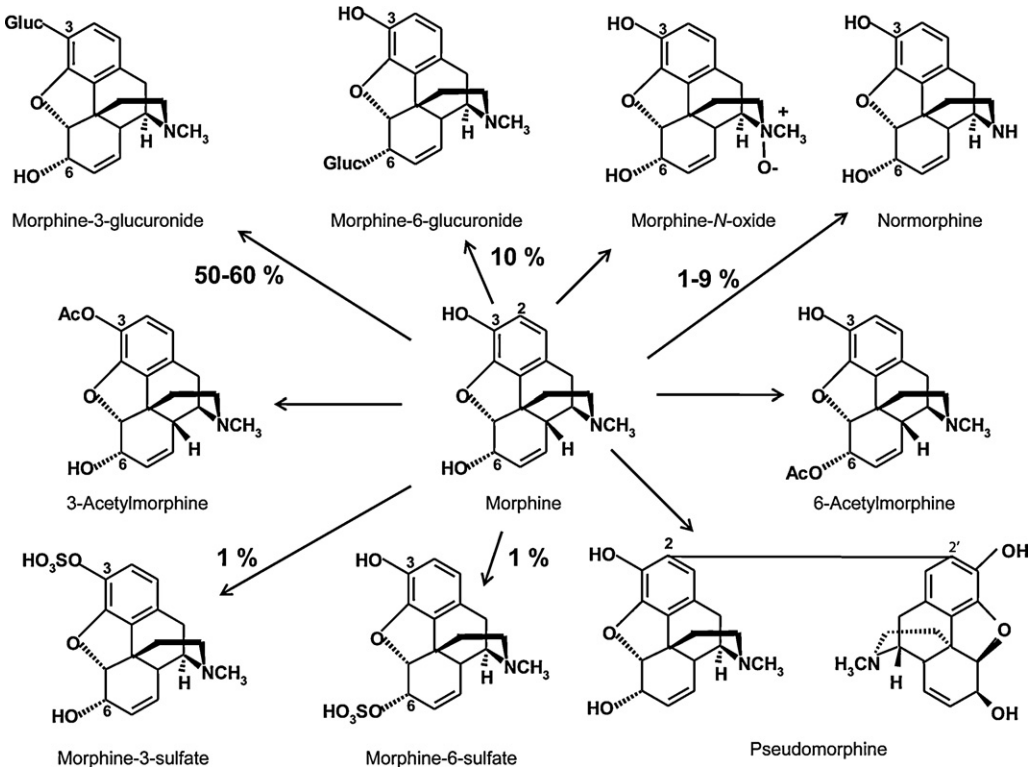

Fig. 1. Metabolic pathways by which morphine undergoes biotransformation in the human body. The majority of the alkaloid is conjugated with glucuronic acid to form morphine-3- and -6-glucuronides. The remainder can be modified to form other metabolites such as sulfate or acetyl esters, normorphine, morphine-N-oxide and pseudomorphine. Adapted from [7].

Much less is known about the activity of other metabolites, since no systematic pharmacological analysis has so far been performed, and only rare single reports on these compounds are available. Lack of information about opiate metabolites does not allow definitive conclusions about their roles in analgesia and in adverse effects of morphine [6,7].

To study the pharmacological properties of various ligands for G protein-coupled receptors, including MOR, we have developed a new real-time approach to monitor G_i-protein activation in intact cells by using biosensors which are based on Förster resonance energy transfer (FRET) [9–11]. In addition, we have developed another technique to monitor β-arrestin recruitment to various receptors by measuring FRET between a receptor and β-arrestin [12,13]. These approaches allow us to study pharmacological properties of different receptors and their ligands in single intact cells with high temporal and spatial resolution [14,15].

Here we used these FRET assays together with radioligand binding studies to perform a systematic analysis of the pharmacological properties of all major morphine metabolites at the human MOR. We found that morphine metabolites exert either strong or weak signaling effects, often comparable to morphine itself, and that some metabolites show a previously unrecognized β-arrestin-biased behavior and are more efficacious in recruiting β-arrestin to the receptor than morphine itself.

2. Materials and methods

2.1. Substances

Morphine, morphine-6-glucuronide, morphine-3-glucuronide and DAMGO ([D-Ala^2, N-MePhe^4, Gly-ol]-enkephalin) were purchased from Sigma–Aldrich (Deisenhofen, Germany). Normorphine, 3-acetylmorphine and 6-acetylmorphine were from Lipomed GmbH (Weil am Rhein, Germany).

Morphine-N-oxide, morphine-3-sulfate, morphine-6-sulfate and pseudomorphine were synthesized as follows. Morphine-N-oxide and pseudomorphine were prepared as described by Garrido et al. [16]. Morphine-3-sulfate was prepared via 6-acetylmorphine as previously described [17] and further sulfonated and deprotected by a procedure described by Mori et al. [18]. Morphine-6-sulfate was synthesized from morphine hydrochloride according to Preechagoon et al. [19]. Except where noted, all other chemicals were obtained from Sigma–Aldrich.

2.2. Plasmids

Human MOR-1A cDNA was obtained from imaGenes GmbH (Berlin, Germany) and subcloned into the pcDNA3 expression vector (Invitrogen) using HindIII and XbaI restriction sites. All fluorescent G_i-protein subunits used to monitor G_i activation by FRET have previously been described [9,10]. Briefly, the rat PTX-insensitive $G_{\alpha i1}$-protein (C351I mutant) was labeled with enhanced yellow fluorescent protein (YFP) between positions 91 and 92. Human $G_{\gamma 2}$ was C-terminally tagged with enhanced cyan fluorescent protein (CFP), and the untagged human $G_{\beta 1}$ cDNA was co-expressed. For the FRET-based measurements of β-arrestin-2 recruitment, the plasmids encoding the human MOR-1A C-terminally tagged with CFP, bovine β-arrestin-2 C-terminally tagged with YFP, and untagged human G protein-coupled receptor kinase (GRK) 2 were used [12].

2.3. Cell culture and transfections

293T cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin and 0.1 mg/ml streptomycin (all from Pan Biotech GmbH, Aidenbach, Germany) at 37 °C and 7% CO_2. Cells were seeded onto poly-D-lysine (Sigma) coated