European Journal of Pharmacology 602 (2009) 250–254

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Molecular and Cellular Pharmacology

Examining the binding properties of MK-0974: A CGRP receptor antagonist for the acute treatment of migraine☆

Eric L. Moore [a,*], Christopher S. Burgey [b], Daniel V. Paone [b], Anthony W. Shaw [b], Yui S. Tang [c], Stefanie A. Kane [a], Christopher A. Salvatore [a]

[a] Merck Research Laboratories, Department of Pain Research, West Point, PA 19486, USA
[b] Merck Research Laboratories, Department of Medicinal Chemistry, West Point, PA 19486, USA
[c] Merck Research Laboratories, DMPK Global Technologies, Rahway, NJ 07065, USA

ARTICLE INFO

Article history:
Received 27 August 2008
Received in revised form 6 November 2008
Accepted 24 November 2008
Available online 3 December 2008

Keywords:
Migraine
CGRP
Telcagepant
Radioligand
MK-0974

ABSTRACT

Calcitonin gene-related peptide (CGRP) is a neuropeptide that plays a key role in the pathophysiology of migraine headache. MK-0974 (telcagepant) is a potent and selective antagonist of the human and rhesus CGRP receptors and is currently in Phase III clinical studies for the acute treatment of migraine. The pharmacology of MK-0974 has been studied extensively, but there has not been a thorough characterization of its binding properties. Here, we characterize the binding of a tritiated analog of MK-0974 on human neuroblastoma (SK-N-MC) membranes and rhesus cerebellum. [^{3}H]MK-0974 displayed reversible and saturable binding to both SK-N-MC membranes and rhesus cerebellum with a K_D of 1.9 nM and 1.3 nM, respectively. Agonists and antagonists of the CGRP receptor displaced [^{3}H]MK-0974 in a concentration-dependent manner in competition binding experiments. Both CGRP and adrenomedullin demonstrated biphasic competition while MK-0974 and the peptide antagonist CGRP(8-37) displaced [^{3}H]MK-0974 in a monophasic fashion. In competitive binding studies with [^{3}H]MK-0974 and CGRP, the fraction of high-affinity binding was reduced significantly by incubating the membranes with GTPγS. In kinetic binding experiments, the off-rate of [^{3}H]MK-0974 was determined to be 0.51 min^{-1} with a half-life of 1.3 min. In conclusion, the radioligand [^{3}H]MK-0974 has proven to be a useful tool for studying the binding characteristics of MK-0974 and has broadened our understanding of this promising molecule.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

Calcitonin gene-related peptide (CGRP) is a 37 amino acid peptide that is produced by alternative splicing of the calcitonin gene (Amara et al., 1982). This peptide exerts its biological effects by binding to a receptor consisting of the G-protein-coupled receptor (GPCR), calcitonin receptor-like receptor (CL receptor) and a single transmembrane protein designated receptor activity-modifying protein (RAMP) 1 (McLatchie et al., 1998). A third protein known as receptor component protein (RCP) is located intracellularly and is required for signal transduction (Evans et al., 2000). In addition to the CGRP receptor, calcitonin receptor-like receptor is also able to form high affinity adrenomedullin receptors by dimerization with either receptor activity-modifying protein 2 (RAMP2) or receptor activity-modifying protein 3 (RAMP3) (McLatchie et al., 1998).

CGRP is widely expressed and exhibits a range of biological functions with the most pronounced being vasodilation. CGRP is the most powerful of the vasodilator transmitters (Brain et al., 1985) and its vasoactive effects have been demonstrated in a variety of blood vessels including those of the cerebral vasculature (Jansen et al., 1992). CGRP-expressing trigeminal nerve endings have been shown to innervate these cerebral blood vessels (Uddman et al., 1985). Multiple lines of evidence point to a possible role for CGRP in migraine headache. CGRP levels in cranial circulation are increased during migraine attacks (Goadsby et al., 1990) and successful treatment of migraine pain with a triptan normalizes CGRP levels (Goadsby and Edvinsson, 1993). In addition, intravenous administration of CGRP to migraineurs results in migraine-like symptoms in some patients (Lassen et al., 1998). More recently, the potent CGRP receptor antagonist olcegepant (BIBN4096BS) demonstrated clinical proof-of-concept for the acute treatment of migraine with intravenous dosing (Olesen et al., 2004).

This body of evidence implicating CGRP in the pathophysiology of migraine led us to initiate our own research program to discover orally bioavailable small-molecule CGRP receptor antagonists that would be suitable for the treatment of migraine. The current standard of care for the treatment of migraine are the 5HT$_{1B/1D}$ receptor agonists which

☆ Authors are employees of Merck & Co., Inc. and potentially own stock and/or hold stock options in the Company.

* Corresponding author. Merck Research Laboratories, WP26A-2000, West Point, PA 19486, USA. Tel.: +1 215 652 2042; fax: +1 215 993 1401.
E-mail address: eric_moore2@merck.com (E.L. Moore).

0014-2999/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2008.11.050

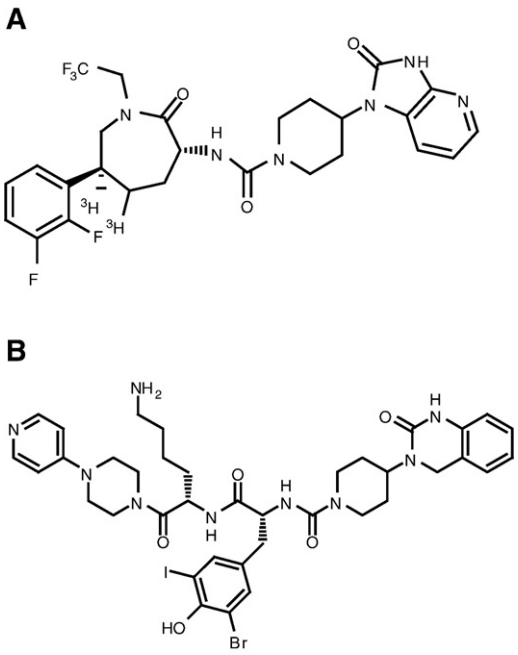

Fig. 1. Chemical structures of A) [^{3}H]MK-0974 and B) Compound 3.

form the triptan class of anti-migraine drugs (Dodick et al., 2004). While adequately safe when used as directed, the triptans are direct vasoconstrictors and are therefore contraindicated for patients with cardiovascular disease (Goadsby et al., 2002). The development of a new class of migraine therapy devoid of these cardiovascular liabilities would be a significant advance in migraine care. We have recently described the identification and pharmacological characterization of MK-0974 (Paone et al., 2007; Salvatore et al., 2008), which is the first orally bioavailable compound in this class to show clinical proof-of-efficacy comparable to triptans in a Phase 2 study (Ho et al., 2008a, 2008b).

The purpose of the study presented here is to characterize the binding properties of the tritiated radioanalog [^{3}H]MK-0974 to human and monkey CGRP receptors.

2. Materials and methods

2.1. Membranes and rhesus cerebellum preparation

SK-N-MC membranes were purchased from Receptor Biology, Inc (Beltsville, MD). Rhesus cerebellum tissue was disrupted in a laboratory homogenizer in cold buffer containing 10 mM HEPES and 5 mM $MgCl_2$. This homogenate was used directly in the binding assays.

2.2. [^{3}H]MK-0974 receptor binding assays

Binding assays were performed by combining [^{3}H]MK-0974, 500 nM Compound 3 (Hershey et al., 2005, Fig. 1B) for non-specific binding, and either 50 µg/well SK-N-MC membrane or 1.5 mg/well rhesus cerebellum homogenate. The assay mixtures were incubated for various times at room temperature in binding buffer (10 mM HEPES, 5 mM $MgCl_2$, 0.2% BSA) in a total volume of 1 ml. Free radioligand was separated from membrane-bound radioligand by filtration through polyethylene imine (0.5%) treated GF/B glass fiber

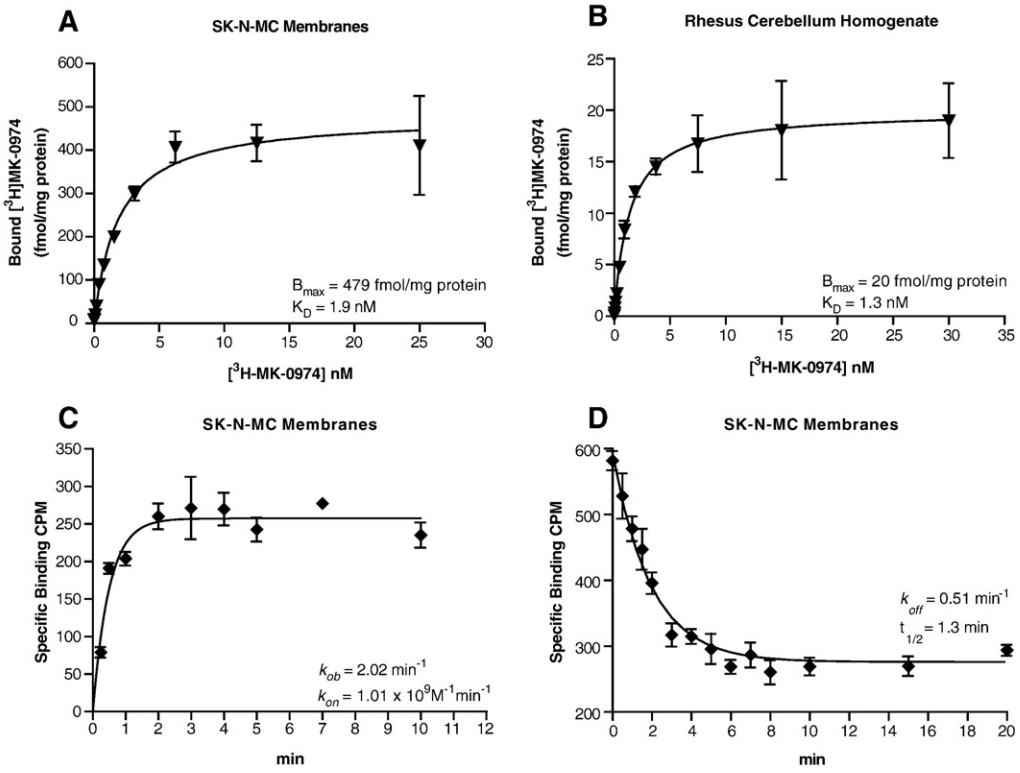

Fig. 2. Saturation binding curves for [^{3}H]MK-0974 on A, 50 µg/well SK-N-MC membranes or B, 1.5 mg/well rhesus cerebellum homogenate. Specific binding was determined by subtracting non-specific binding from the total binding curve. Symbols and error bars represent mean and standard deviation from 5 separate titrations. Panel C shows the association kinetics of [^{3}H]MK-0974 binding to SK-N-MC membranes at room temperature. 1.5 nM [^{3}H]MK-0974 was added to 50 µg/well SK-N-MC membranes and binding was monitored over a period of 10 min. This is a representative graph of three separate experiments. Symbols and error bars represent the mean and standard deviation of 5 points. Panel D shows the dissociation kinetics of [^{3}H]MK-0974 binding to SK-N-MC membranes. 1.5 nM [^{3}H]MK-0974 was added to 50 µg/well SK-N-MC membranes and incubated 3 h at room temperature. Dissociation was initiated by the addition of 500 nM compound 3 and monitored over a period of 20 min. Symbols and error bars represent the mean and standard deviation from two separate experiments.

Download English Version:

https://daneshyari.com/en/article/2534506

Download Persian Version:

https://daneshyari.com/article/2534506

Daneshyari.com