Neuroscience 324 (2016) 151–162

A NOVEL 3-(4,5-DIPHENYL-1,3-OXAZOL-2-YL)PROPANAL OXIME COMPOUND IS A POTENT TRANSIENT RECEPTOR POTENTIAL ANKYRIN 1 AND VANILLOID 1 (TRPA1 AND V1) RECEPTOR ANTAGONIST

M. PAYRITS,[a,b†] É. SÁGHY,[a,b†] P. MÁTYUS,[d] A. CZOMPA,[d] R. LUDMERCZKI,[d] R. DEME,[d] Z. SÁNDOR,[a] ZS. HELYES[a,b,c‡] AND É. SZŐKE[a,b,c*‡]

[a] *Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Pécs, Pécs-7624, Szigeti str. 12., Hungary*

[b] *Szentágothai Research Centre, University of Pécs, Pécs-7624, Ifjúság str. 20., Hungary*

[c] *MTA-PTE Chronic Pain Research Group, Pécs-7624, Szigeti str. 12., Hungary*

[d] *Department of Organic Chemistry, University of Semmelweis, Budapest-1092, Hőgyes Endre str. 7., Hungary*

Abstract—Transient Receptor Potential Ankyrin 1 and Vanilloid 1 (TRPA1, TRPV1) ion channels expressed on nociceptive primary sensory neurons are important regulators of pain and inflammation. TRPA1 is activated by several inflammatory mediators including formaldehyde and methylglyoxal that are products of the semicarbazide-sensitive amine-oxidase enzyme (SSAO). SZV-1287 is a new 3-(4,5-diphenyl-1,3-oxazol-2-yl)propanal oxime SSAO inhibitor, its chemical structure is similar to other oxime derivatives described as TRPA1 antagonists. Therefore, we investigated its effects on TRPA1 and TRPV1 receptor activation on the cell bodies and peripheral terminals of primary sensory neurons and TRPA1 or TRPV1 receptor-expressing cell lines. Calcium influx in response to the TRPA1 agonist allyl-isothiocyanate (AITC) (200 μM) and the TRPV1 stimulator capsaicin (330 nM) in rat trigeminal neurons or TRPA1 and TRPV1 receptor-expressing cell lines was measured by microfluorimetry or radioactive $^{45}Ca^{2+}$ uptake experiments. Calcitonin gene-related peptide (CGRP) release as the indicator of 100 μM AITC – or 100 nM capsaicin-induced peripheral sensory nerve terminal activation was measured by radioimmunoassay. SZV-1287 (100, 500 and 1000 nM) exerted a concentration-dependent significant inhibition on both AITC- and capsaicin-evoked calcium influx in trigeminal neurons and TRPA1 or TRPV1 receptor-expressing cell lines. It also significantly inhibited the TRPA1, but not the TRPV1 activation-induced CGRP release from the peripheral sensory nerve endings in a concentration-dependent manner. In contrast, the reference SSAO inhibitor LJP 1207 with a different structure had no effect on TRPA1 or TRPV1 activation in either model system. This is the first evidence that our novel oxime compound SZV-1287 originally developed as a SSAO inhibitor has a potent dual antagonistic action on TRPA1 and TRPV1 ion channels on primary sensory neurons.

Key words: Transient Receptor Potential Ankyrin 1 and Vanilloid 1 receptors, capsaicin, allyl-isothiocyanate, SZV-1287, semicarbazide-sensitive amine-oxidase, sensory neuron.

INTRODUCTION

The Transient Receptor Potential (TRP) cation channels, including Ankyrin repeat domain 1 (TRPA1) and Vanilloid type 1 (TRPV1) are important regulators of nociceptive and inflammatory processing (Akopian et al., 2007; Salas et al., 2009). Besides a variety of exogenous TRPA1 agonists, such as cinnamaldehyde, allyl-isothiocyanate (AITC in mustard oil) and allicin, there are a lot of endogenous activators produced by tissue injury and inflammation, e.g. formaldehyde and the reactive cytotoxic metabolite methylglyoxal which can activate the TRPA1 channel (Bandell et al., 2004; Jordt et al., 2004; Bautista et al., 2005; Macpherson et al., 2005, 2007; McNamara et al., 2007; Trevisani et al., 2007; Wang et al., 2008; Eberhardt et al., 2012). In addition, mediators of oxidative stress, cold and mechanical stimuli also gate the TRPA1 (Story et al., 2003; Andersson et al., 2008). Besides the two classical vanilloid type agonists, capsaicin and resiniferatoxin, TRPV1 is activated by several highly lipophylic compounds as endogenous arachidonic acid or other fatty acid metabolites like

*Correspondence to: É. Szőke, Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Pécs, Pécs-7624, Szigeti str. 12., Hungary. Tel: +36-72-536217x5386; fax.: +36-72-536218.

E-mail addresses: payrits.maja@gmail.com (M. Payrits), saghyeva@gmail.com (É. Sághy), matyus.peter@pharma.semmelweis-univ.hu (P. Mátyus), czompa.andrea@pharma.semmelweis-univ.hu (A. Czompa), ludmerczki.robert@pharma.semmelweis-univ.hu (R. Ludmerczki), deme.ruth@pharma.semmelweis-univ.hu (R. Deme), zoltan.sandor@aok.pte.hu (Z. Sándor), zsuzsanna.helyes@aok.pte.hu (Zs. Helyes), eva.szoke@aok.pte.hu (É. Szőke).

† M. Payrits and É. Sághy are contributed equally to this work as first authors.

‡ Zs. Helyes and É. Szőke are contributed equally to this work as senior authors.

Abbreviations: AITC, allyl-isothiocyanate; CGRP, calcitonin gene-related peptide; CHO, Chinese hamster ovary cell line; COX, cyclooxygenase; CZP, capsazepine; D-MEM, Dulbecco's-Modified Eagle Medium; DMSO, dimethyl sulfoxide; ECS, extracellular solution; LJP 1207, N′-(2-Phenylallyl)hydrazine hydrochloride; NGF, nerve growth factor; PBS, phosphate-buffered solution; SSAO, semicarbazide-sensitive amine-oxidase; SZV-1287, 3-(4,5-diphenyl-1,3-oxazol-2-yl)propanal oxim; TG, trigeminal ganglion; TRPV1, Transient Receptor Potential Vanilloid 1; TRPA1, Transient Receptor Potential Ankyrin Repeat Domain 1; TRPM8, Transient Receptor Potential Melastatin 8; $[Ca^{2+}]_i$, intracellular free calcium concentration.

http://dx.doi.org/10.1016/j.neuroscience.2016.02.049
0306-4522/

anandamide, N-oleoyldopamine (Smart et al., 2000; Hwang et al., 2000; Bianchi et al., 2006). Protons (pH < 6.0) and noxious heat (> 43 °C) are also able to directly open this channel (Welch et al., 2000; Myers et al., 2008).

Semicarbazide-sensitive amine-oxidase (SSAO) also known as vascular adhesion protein-1 is expressed predominantly in vascular smooth muscle cells, and can be found as membrane-bound and circulating forms (Lyles, 1996). It catalyzes oxidative deamination of primary amines, resulting in the production of aldehydes, as well as the release of ammonia and hydrogen peroxide (Buffoni and Ignesti, 2000; O'Sullivan et al., 2004). It produces tissue irritants, such as formaldehyde and methylglyoxal, which are TRPA1 channel activators. Formaldehyde and methylglyoxal activate TRPA1 by covalent modification of cysteine and lysine residues in the N-terminal intracellular domain of the receptor channel (Macpherson et al., 2007; McNamara et al., 2007; Eberhardt et al., 2012). The products of the SSAO, such as formaldehyde and methylglyoxal, are potentially more toxic than the original substrates. Acrolein produced by the metabolism of the xenobiotic allylamine by SSAO causes vascular toxicity (Buffoni and Ignesti, 2000; O'Sullivan et al., 2004). Simultaneous inhibition of the potential toxic actions of these tissue irritants and TRPA1/TRPV1 antagonism is likely to result in a more favorable therapeutical potential. A link between SSAO activity and TRPA1 stimulation has not yet been thought of. The roles of SSAO and the effects of its inhibitors were investigated in inflammation, angiogenesis, cancer and diabetes (Salmi et al., 1993; O'Sullivan et al., 2004; Noda et al., 2009; Enzsöly et al., 2011), but there are no data regarding its function in pain and nociception. SZV-1287 is our new 3-(4,5-diphenyl-1,3-oxazol-2-yl) propanal oxime SSAO inhibitor (Fig. 1) developed from the oxime analog of the cyclooxygenase (COX) inhibitor oxaprozin (Tábi et al., 2013).

It exerts both SSAO and COX inhibitory actions, and inhibits both acute and chronic inflammation in rats more effectively than the reference SSAO inhibitor, LJP 1207 (N′-(2-Phenylallyl)hydrazine hydrochloride; Fig. 1; Wang et al., 2006). Since the chemical structure of SZV-1287 is similar to other oxime derivatives shown to be selective TRPA1 antagonists. Our compound contains the same oxime residue which is present in AP18 ((Z)-4-(4-chlorophenyl)-3-methylbut-3-en-2-oxime) and A-967079. (Baraldi et al., 2010; DeFalco et al., 2010; Chen et al., 2011). We investigated the effects of SZV-1287 on TRPA1 receptor activation-induced responses of primary sensory neuronal cell bodies and peripheral terminals. HC030031 (Fig. 1), the frequently used reference TRPA1 antagonist (Eid et al., 2008) and a conventional SSAO inhibitor LJP 1207 (Wang et al., 2006) of different structure (Salter-Cid et al., 2005; Tábi et al., 2013) served as reference compounds for comparison. TRPA1 and TRPV1 show structural and functional similarities, they are co-localized on the capsaicin-sensitive primary sensory neurons (Story et al., 2003; Caterina and Park, 2006; Szolcsányi, 2008) and there is an interaction between these ion channels (Akopian et al., 2007;

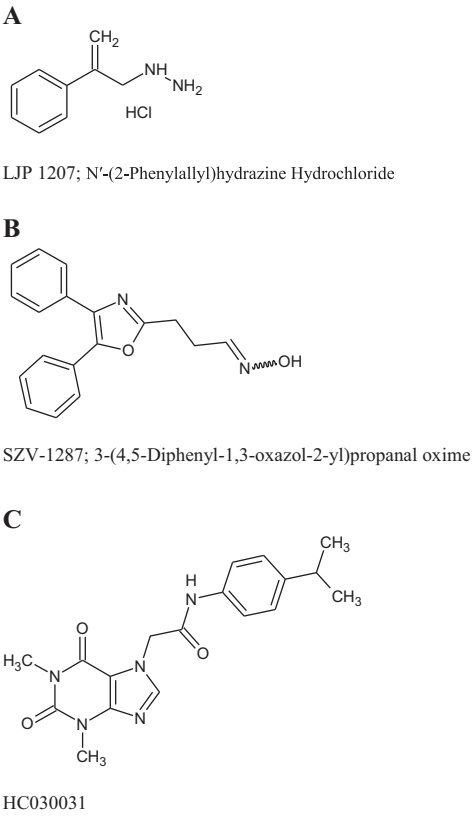


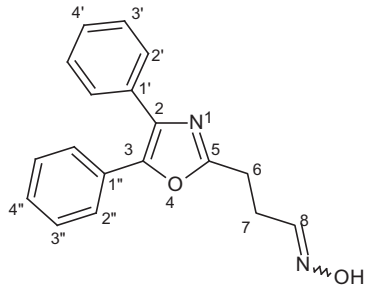
Fig. 1. Structure of (A) LPJ-1207 (B) SZV-1287 (C) HC030031.

Salas et al., 2009; Patil et al., 2010). Therefore, we investigated the effects of these compounds also on capsaicin-evoked responses in the same model systems.

EXPERIMENTAL PROCEDURES

Synthesis of 3-(4,5-diphenyl-1,3-oxazol-2-yl)propanal oxime (SZV-1287)

Synthesis of SZV-1287 ($C_{18}H_{16}N_2O_2$; MW = 292.33) was effectively accomplished by the direct condensation of 3-(4,5-diphenyl-1,3-oxazol-2-yl)propanal (Pridgen et al., 1984) with hydroxylamine in the following way.



(The numbering relates to NMR assignment.)

In a two-necked flask 3-(4,5-diphenyl-1,3-oxazol-2-yl) propanal (4.65 g, 16.78 mmol) and ethanol (80 ml) were measured under argon atmosphere. To the solution, a