European Journal of Pharmacology 837 (2018) 137–144

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Molecular and cellular pharmacology

Pharmacological characterization of the orexin/hypocretin receptor agonist Nag 26

Maiju K. Rinne[a,b], Teppo O. Leino[b], Ainoleena Turku[a,b], Pauli M. Turunen[a,c], Yana Steynen[b], Henri Xhaard[b], Erik A.A. Wallén[b], Jyrki P. Kukkonen[a,c,*]

[a] Biochemistry and Cell Biology, Department of Veterinary Biosciences, Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland
[b] Drug Research Program, Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, Helsinki, Finland
[c] Physiology, Institute of Biomedicine, Faculty of Medicine, University of Helsinki, Helsinki, Finland

ARTICLE INFO

Keywords:
Orexin
Calcium
Phospholipase C
cAMP
Cell death
ERK

ABSTRACT

One promising series of small-molecule orexin receptor agonists has been described, but the molecular pharmacological properties, i.e. ability and potency to activate the different orexin receptor-regulated signal pathways have not been reported for any of these ligands. We have thus here assessed these properties for the most potent ligand of the series, 4′-methoxy-*N*,*N*-dimethyl-3′-[*N*-(3-{[2-(3-methylbenzamido)ethyl]amino}phenyl sulfamoyl]-(1,1′-biphenyl)-3-carboxamide (Nag 26). Chinese hamster ovary-K1 cells expressing human orexin receptor subtypes OX_1 and OX_2 were used. Ca^{2+} elevation and cell viability and death were assessed by fluorescent methods, the extracellular signal-regulated kinase pathway by a luminescent Elk-1 reporter assay, and phospholipase C and adenylyl cyclase activities by radioactive methods. The data suggest that for the G_q-dependent responses, Ca^{2+}, phospholipase C and Elk-1, Nag 26 is a full agonist for both receptors, though of much lower potency. However, saturation was not always reached for OX_1, partially due to Nag 26's low solubility and partially because the response decreased at high concentrations. The latter occurs in the same range as some reduction of cell viability, which is independent of orexin receptors. Based on the EC_{50}, Nag 26 was OX_2-selective by 20–200 fold in different assays, with some indication of biased agonism (as compared to orexin-A). Nag 26 is a potent orexin receptor agonist with a largely similar pharmacological profile as orexin-A. However, its weaker potency (low–mid micromolar) and low water solubility as well as the non-specific effect in the mid-micromolar range may limit its usefulness under physiological conditions.

1. Introduction

Orexin-A and -B are hypothalamic neuropeptides that have been most strongly associated with regulation of sleep and wakefulness (reviewed in Li et al., 2017; Scammell et al., 2017). This is most clearly seen in narcolepsy; death of orexinergic neurons is thought to lie behind human narcolepsy (Nishino et al., 2000), and narcoleptic phenotype is seen in animals upon genetic destruction of orexinergic neurons, orexin peptides or orexin receptors, or toxic destruction of orexin target neurons (Chemelli et al., 1999; Lin et al., 1999; Hara et al., 2001; Gerashchenko et al., 2001; Willie et al., 2003). The orexin system has also been associated with other central physiological and pathophysiological functions, such as metabolic regulation, feeding, stress response, addiction and pain gating (reviewed in Kukkonen, 2013; Baimel et al., 2015), and orexin receptor polymorphisms have been and are investigated with respect to additional indications such as anxiety and depression (reviewed in Thompson et al., 2014). It can thus be assumed that both orexin receptor antagonists and agonists might be useful in different types of disorders. Orexin receptor antagonist discovery is currently mainly focusing on the development of hypnotics (reviewed in Roecker et al., 2016); one such drug, suvorexant, is already on the market in the US and Japan. Orexin receptor agonists, on the other hand, would find an obvious use in narcolepsy − a concept that has been proven in rodents (Mieda et al., 2004; Kantor et al., 2013; Irukayama-Tomobe et al., 2017) − as well as likely in other disorders of increased sleep and reduced vigilance. Other apparent, though thus far less substantiated, uses of the agonists would be in some metabolic disorders and some cancers; orexin receptor stimulation can also stimulate programmed cell death of some cancer cells (Rouet-Benzineb et al., 2004; Ammoun et al., 2006; Voisin et al., 2006, 2011).

While orexin antagonist development has been very active and successful, little has happened on the agonist side. Only one potent

* Correspondence to: Physiology, Institute of Biomedicine, Faculty of Medicine, POB 63, FI-00014 University of Helsinki, Finland.
E-mail address: jyrki.kukkonen@helsinki.fi (J.P. Kukkonen).

https://doi.org/10.1016/j.ejphar.2018.09.003
Received 23 April 2018; Received in revised form 31 August 2018; Accepted 4 September 2018
Available online 05 September 2018
0014-2999/ © 2018 Elsevier B.V. All rights reserved.

series of small molecular weight orexin receptor agonists has been described (Nagahara et al., 2015); the other reported agonists appear to have low potency and possible low selectivity (see, e.g. Turku et al., 2016; Turku et al., 2017). The pharmacological properties of even the promising series reported in (Nagahara et al., 2015) are barely known. We thus decided to characterized the most potent ligand of the series, 4′-methoxy-*N*,*N*-dimethyl-3′-[*N*-(3-{[2-(3-methylbenzamido)ethyl] amino}phenyl) sulfamoyl]-(1,1′-biphenyl)-3-carboxamide (compound 26; here referred as Nag 26), in the current study. The ligand was synthesized and its activity and potency with respect to a number of different responses, involving the three classical G-protein pathways, was assessed for both human orexin receptor subtypes expressed in Chinese hamster ovary K1 (CHO) cells.

2. Materials and methods

2.1. Materials

Human orexin-A was from NeoMPS (Strasbourg, France), 2-(1-[3-dimethylaminopropyl]-1*H*-indol-3-yl)-3-(1*H*-indol-3-yl)-maleimide (GF109203X), *N*-biphenyl-2-yl-1-{[(1-methyl-1*H*-benzimidazol-2-yl) sulfanyl]acetyl}-L-prolinamide (TCS 1102), (2*Z*,3*Z*)-bis({amino[(2-aminophenyl)sulfanyl]methylidene}) butanedinitrile (U0126), thapsigargin and pertussis toxin from Tocris Bioscience (Bristol, UK), and cholera toxin, forskolin, staurosporin and 3-isobutyl-1-methylxanthine from Sigma-Aldrich. Hoechst 33342 [2′-(4-ethoxyphenyl)-5-(4-methyl-1-piperazinyl)-1*H*,1′*H*-2,5′-bibenzimidazole; here abbreviated as Hoechst] was from Molecular Probes/Life Technologies (Carlsbad, CA, USA), PrestoBlue and 4-[(3-methyl-1,3-benzoxazol-2(3 *H*)-ylidene)methyl]-1-[3-(trimethylammonio)propyl] quinolinium diiodide (YO-PRO-1 iodide, here abbreviated as YO-PRO) from Thermo Fisher Scientific Finland (Espoo, Finland) and L-threonine,(3 *R*)-*N*-acetyl-3-hydroxy-L-leucyl-(a*R*)-a-hydroxybenzenepropanoyl-2,3-idehydro-*N*-methylalanyl-L-alanyl-*N*-methyl-L-alanyl-(3 *R*)-3-[[(2 *S*,3 *R*)-3-hydroxy-4-methyl-1-oxo-2-[(1-oxopropyl)amino]pentyl]oxy]-L-leucyl-*N*,*O*-dimethyl-,(7→1)-lactone (9CI) (UBO-QIC a.k.a. FR900359) from the Institute of Pharmaceutical Biology, University of Bonn (Bonn, Germany; http://www.pharmbio.uni-bonn.de/signaltransduktion/quote). *Myo*-[2–^{3}H] inositol (PT6–271) and [2,8-^{3}H]adenine were from PerkinElmer Life and Analytical Sciences (Waltham, MA, USA).

Nag 26 was synthesized applying the method by Nagahara et al. (2015). The identity of compound was verified by high-resolution mass spectrometry and ^{1}H and ^{13}C NMR spectroscopy; these data are in accordance with the literature. Purity was > 95% as assessed by ultra performance liquid chromatography. Details of the analysis are as follows: White, amorphous solid. ^{1}H NMR (400 MHz, CD_3CN) δ 8.02 (d, J = 2.4 Hz, 1 H), 7.80–7.78 (m, 2 H), 7.61–7.57 (m, 2 H), 7.54–7.51 (m, 2 H), 7.41 (t, J = 7.8 Hz, 1 H), 7.34–7.27 (m, 3 H), 7.23 (t, J = 4.8 Hz, 1 H), 7.17 (d, J = 8.4 Hz, 1 H), 6.91 (t, J = 8.0 Hz, 1 H), 6.43 (t, J = 2.2 Hz, 1 H), 6.36–6.33 (m, 1 H), 6.29–6.26 (m, 1 H), 4.71 (s, 1 H), 4.02 (s, 3 H), 3.48 (q, J = 6.0 Hz, 2 H), 3.16–3.15 (m, 2 H), 3.02 (s, 3 H), 2.91 (s, 3 H), 2.35 (s, 3 H). ^{13}C NMR (101 MHz, CD_3CN) δ 171.7, 168.6, 157.1, 150.4, 139.9, 139.4, 139.3, 138.6, 135.4, 134.3, 133.2, 133.0, 130.7, 130.0, 130.0, 129.3, 128.7, 128.4, 127.6, 126.9, 125.9, 125.1, 114.1, 110.3, 109.6, 104.5, 57.2, 44.0, 39.8, 39.7, 35.4, 21.4. High-resolution mass spectrometry (electrospray ionization quadrupole time-of-flight mass spectrometry) *m/z*: Calculated for $[M+H]^+$ $C_{32}H_{35}N_4O_5S$ 587.2328; Found 587.2325.

2.2. Cell culture and media

CHO-hOX_1 and -hOX_2 cells (Lund et al., 2000; Ammoun et al., 2003) as well as ctrl CHO-K1 cells (not expressing orexin receptors; ctrl CHO cells) were cultured in Ham's F12 medium (Gibco/Life Technologies, Paisley, UK) + supplements on plastic cell culture dishes (56 cm^2 bottom area; Greiner Bio-One GmbH, Frickenhausen, Germany) as described in (Jäntti et al., 2012). Different types of multi-well plates were used for the assays: black, clear-bottom half-area Cellstar μClear 96-well cell culture plates (Greiner Bio-One GmbH) for Ca^{2+} measurements; black, clear-bottom Cellstar μClear 96-well cell culture plates (Greiner) for cell death assays; and clear Cellstar 48- or 96-well cell culture plates (Greiner) for phospholipase C and adenylyl cyclase assays. All multi-well plates were coated with polyethyleneimine (25 μg/ml for 1 h at 37 °C; Sigma-Aldrich, St. Louis, MO, USA). Cells were for the adenylyl cyclase experiments pretreated for 20 h with cholera toxin (1000 ng/ml) or for 36 h with pertussis toxin (300 ng/ml).

Hepes-buffered medium (HBM) was used as the basic experimental medium. It was composed of 137 mM NaCl, 5 mM KCl, 1.2 mM $MgCl_2$, 0.44 mM KH_2PO_4, 4.2 mM $NaHCO_3$, 1 mM $CaCl_2$, 10 mM glucose, 20 mM HEPES, and adjusted to pH 7.4 with NaOH.

2.3. Ca^{2+} elevation

Ca^{2+} elevations were measured as described before (Turku et al., 2017). Briefly, 1.5×10^4 cells per well were plated on black, clear bottom half-area 96-well plates. Twenty-four hours later, cell culture medium was removed and the cells were exposed to the loading solution composed of FLIPR Calcium 4 Assay Kit (Molecular Devices, Sunnyvale, CA, USA) dissolved in and diluted with HBM + 1 mM probenecid, for 60 min at 37 °C. Intracellular Ca^{2+} levels were measured as fluorescence changes (excitation at 485 nm, emission at 525 nm) at 37 °C using a FlexStation 3 fluorescence plate reader (Molecular Devices). A recording was made approximately every 1.3 s. Each well was measured for 150 s with 30 s of baseline before stimulation (see Fig. 1AB). Antagonists were manually added in the wells during the probe incubation (30 min before the measurement).

2.4. Phospholipase C activity

Phospholipase C activity was measured as described before (Turku et al., 2017). Briefly, 1.1×10^4 or 2.4×10^4 cells per well were plated on clear 96- or 48-well plates, respectively. Twenty-four hours later, they were labelled with 3 μCi/ml [^{3}H]inositol for 20 h. The medium was removed, and the cells were incubated in HBM supplemented with 10 mM LiCl for 30 min at 37 °C; also the possible inhibitors, TCS 1102 and L-threonine, (3 *R*)-*N*-acetyl-3-hydroxy-L-leucyl-(a*R*)-a-hydroxybenzenepropanoyl-2,3-idehydro-*N*-methylalanyl-L-alanyl-*N*-methyl-L-alanyl-(3 *R*)-3-[[(2 *S*,3 *R*)-3-hydroxy-4-methyl-1-oxo-2-[(1-oxopropyl)amino] pentyl]oxy]-L-leucyl-*N*,*O*-dimethyl-,(7→1)-lactone (9CI) (UBO-QIC), were included in this incubation. They were then stimulated with orexin-A or Nag 26 for 30 min, after which the medium was rapidly removed and the reactions stopped with ice-cold perchloric acid and freezing. The samples were thawed and neutralized, and the insoluble fragments spun down. The total inositol phosphate fraction of the supernatants was isolated by anion-exchange chromatography, and the radioactivity determined by scintillation counting (HiSafe 3 scintillation cocktail and Wallac 1415 liquid scintillation counter; PerkinElmer).

2.5. Adenylyl cyclase activity

Adenylyl cyclase activity was measured as described before (Turku et al., 2017). Briefly, 2.4×10^4 (cholera toxin-treated) or 3.0×10^4 (pertussis toxin-treated) cells per well were plated on clear 48-well plates. The cells were treated with pertussis toxin after 8 h or with cholera toxin after 24 h. Forty-eight hours after the plating, the cells were labelled with 5 μCi/ml [^{3}H]adenine for 2 h. After the labelling, the cells were washed once with phosphate-buffered saline. HBM, supplemented with 500 μM 3-isobutyl-1-methylxanthine (a cyclic nucleotide phosphodiesterase inhibitor) and 3 μM GF109203X (a protein kinase C inhibitor), was added to the cells. The cells were incubated for 30 min at 37 °C before adding the stimulants (forskolin ± orexin-A or Nag 26). After an additional 10-min incubation at 37 °C, the medium was

Download English Version:

https://daneshyari.com/en/article/10143284

Download Persian Version:

https://daneshyari.com/article/10143284

Daneshyari.com