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ScienceDirect
European Journal of Pharmacology 580 (2008) 1 – 11
www.elsevier.com/locate/ejphar

$GABA_A$ receptor pharmacology of fluorinated derivatives of the novel sedative-hypnotic pyrazolopyrimidine indiplon

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Received 31 May 2007; received in revised form 8 October 2007; accepted 15 October 2007
Available online 22 October 2007

Abstract

The function of γ-aminobutyric acid type A receptors ($GABA_A$ receptors) is enhanced by various clinically important drugs including benzodiazepines that act on an allosteric site formed at the interface between the α and γ subunits. In contrast to classical benzodiazepines, the novel pyrazolopyrimidine indiplon (*N*-methyl-*N*-{3-[7-(thiophene-2-carbonyl)-1,5,9-triazabicyclo[4.3.0]nona-2,4,6,8-tetraen-2-yl]phenyl}acetamide; *N*-methyl-*N*-{3-[3-(thiophene-2-carbonyl)-pyrazolo[1,5-*a*]pyrimidine-7-yl]phenyl}-acetamide) demonstrates relative binding selectivity for the α1 subunit containing receptor subtypes, which are the most frequently expressed in the mammalian central nervous system. To investigate the pharmacological properties at $GABA_A$ receptors and to promote the development of α1 subunit selective radiotracers for positron emission tomography imaging, we have started with the evaluation of various fluorinated indiplon derivatives. Binding affinities were determined in homogenates from newborn and adult rats suggesting an α1 preference of the reference compounds indiplon, zaleplon as well as for all newly synthesized indiplon derivatives. In homogenated cerebellar tissue obtained from adult rat brain, known to primarily express α1 containing $GABA_A$ receptors, the high affinity of the basic indiplon structure was only slightly affected by an elongation of the alkyl substituent of the amide N from methyl (indiplon; K_i 3.1 nM) via ethyl (**2a**, *N*-(2-fluoro-ethyl)-*N*-{3-[3-(thiophene-2-carbonyl)-pyrazolo[1,5-*a*]pyrimidine-7-yl]phenyl}-acetamide; K_i 5.4 nM) to propyl (**2b**, *N*-(3-fluoro-propyl)-*N*-{3-[3-(thiophene-2-carbonyl)-pyrazolo[1,5-*a*]pyrimidine-7-yl]phenyl}-acetamide; K_i 2.4 nM). Whole cell patch-clamp recordings at neuronal and recombinant $GABA_A$ receptors indicated that the fluorinated derivatives **2a** and **2b** have a high potency at α1β3γ2L isoforms comparable to indiplon (EC_{50}: 105, 158, and 81 nM, respectively), with **2b** displaying the most pronounced efficacy at α3β3γ2L subtypes. In conclusion, the affinity profiles and functional properties of the newly synthesised fluorinated indiplon derivatives make compounds **2a** and **2b** suitable for the development of [^{18}F]-labelled ligands at $GABA_A$ receptors containing the α1 subunit.

Keywords: $GABA_A$ receptor; α1 Subunit; Non-benzodiazepine; Fluorinated indiplon derivative; Radioligand binding assay; Electrophysiology

1. Introduction

Fast inhibitory neurotransmission in the brain is mediated by synaptic γ-aminobutyric acid type A receptors ($GABA_A$ receptors) (Macdonald and Olsen, 1994). $GABA_A$ receptor isoforms, the majority of which are synaptic αβγ heteromers, are composed of five subunits (2α, 2β, 1γ) that form a transmembrane chloride ion channel (Macdonald and Olsen, 1994; Barnard et al., 1998) and are differently expressed during

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doi:10.1016/j.ejphar.2007.10.016

postnatal CNS development (Fritschy et al., 1994). Isoforms containing the α1 subunit constitute approximately 60% of the $GABA_A$ receptor population, primarily distributed in the thalamus and cortical areas, while subtypes with α2 (15–20%), α3 (10–15%) or α5 subunits (<5%) are less abundant in the adult brain (Whiting et al., 1999; Möhler et al., 2002, Ebert et al., 2006). Both, this heterogeneous expression and the subunit assembly confer specific physiological and pharmacological properties of $GABA_A$ receptors (Sigel et al., 1990; Macdonald and Olsen, 1994). Several studies have revealed that α1 containing isoforms mediate the ability of benzodiazepine site ligands to yield sedative, anxiolytic, and amnestic effects, while receptors containing α2 or α3 subunits influence anxiolytic pathways (McKernan and Whiting, 1996; Sieghart et al., 1999; McKernan et al., 2000; Möhler et al., 2002; Mendelson et al., 2004; Dias et al., 2005).

$GABA_A$ receptor function is enhanced by various clinically important drugs including benzodiazepines that act on an allosteric site formed by α and γ subunits (Macdonald and Olsen, 1994; Wafford 2005). In contrast to classical benzodiazepines (e.g. diazepam, triazolam), the non-benzodiazepine hypnotics zolpidem and zaleplon, which also bind to the benzodiazepine site, demonstrate relative α1 subunit selectivity regarding binding and potency (Sanna et al., 2002; Sullivan et al., 2004; Ebert et al., 2006; Petroski et al., 2006). The novel hypnotic non-benzodiazepine indiplon, a pyrazolopyrimidine based compound, has a short pharmacological half-life (~1.5 h), produces dose-dependent motor performance deficits (Voss et al., 2003), displays a high affinitiy for α1 containing isoforms, and awaits approval for the treatment of insomnia by the FDA (Foster et al., 2004; Sullivan et al., 2004; Petroski et al., 2006; Ebert et al., 2006).

Besides insomnia, an impaired GABAergic neurotransmission has been implicated in various neurological and psychiatric diseases, such as epilepsy, anxiety disorders, and alcoholism (Armijo et al., 2005; Steriade, 2005; Rout, 2005; Zwanzger and Rupprecht, 2005; Krystal et al., 2006) which has promoted the development and use of radiotracers for positron emission tomography (PET) imaging of $GABA_A$ receptors. [^{11}C]flumazenil has been regarded as the ligand of choice although the rapid metabolism, the lack of subtype selectivity, and the short half-life of ^{11}C ($t_{1/2}$ ~20 min) limit its clinical use (Goethals et al., 2003; Bartenstein, 2004; Katsifis and Kassiou, 2004). Biological evaluation of new ^{18}F-labelled ($t_{1/2}$ ~110 min) flumazenil derivatives revealed their suitability as PET imaging agents (Mitterhäuser et al., 2004; Chang et al., 2005; Ryzhikov et al., 2005), however, these compounds still display a lack of subtype selectivity. To overcome this problem and to study the pharmacological properties at $GABA_A$ receptors, we have started with the development of fluorinated indiplon derivatives (Hoepping et al., 2007, in press).

Data on the affinity as well as subunit preference were obtained on native $GABA_A$ receptor isoforms. Competitive radioligand binding studies in newborn and adult rats were performed to investigate $GABA_A$ receptor affinities of indiplon, various novel fluorinated indiplon derivatives, and zaleplon. Due to the unique postnatal expression pattern of each $GABA_A$ receptor subunit, brains of newborn rats contain mainly α2/α3 $GABA_A$ receptors while α1 containing subtypes constitute the largest population of $GABA_A$ receptors in the adult cerebellum (Laurie et al., 1992).

For electrophysiological analyses of the test compounds we used whole cell patch-clamp recordings to measure pharmacological effects in HEK293T cells transiently transfected with recombinant $GABA_A$ receptors containing different α subunits and in human NT2 neurons. Differentiated NT2 cells extend dendritic and axonal processes, and express human neuron-specific cell surface, cytoskeletal, and secretory markers as well as functional $GABA_A$ receptors (Pleasure et al., 1992; Neelands et al., 1998). We show that the fluorinated indiplon derivatives **2a** and **2b** have a high affinity, specificity, and potency at neuronal and recombinant α1 containing $GABA_A$ receptors comparable to indiplon, while **2b** displays the most pronounced efficacy at isoforms containing α3 subunits.

2. Materials and methods

2.1. Chemistry

The details about synthesis and analytics of indiplon (*N*-methyl-*N*-{3-[3-(thiophene-2-carbonyl)-pyrazolo[1,5-a]pyrimidine-7-yl]phenyl}-acetamide) and the fluorinated indiplon derivatives **1a** (2-fluoro-*N*-methyl-*N*-(3-[3-(thiophene-2-carbonyl)-pyrazolo[1,5-a]pyrimidine-7-yl]-phenyl)-acetamide), **1b** (4-fluoro-*N*-methyl-*N*-{3-[3-(thiophene-2-carbonyl)-pyrazolo [1,5-a]pyrimidine-7-yl]phenyl}-benzamide), **2a** (*N*-(2-fluoroethyl)-*N*-{3-[3-(thiophene-2-carbonyl)-pyrazolo[1,5-a]pyrimidine-7-yl]phenyl}-acetamide), **2b** (*N*-(3-fluoro-propyl)-*N*-{3-[3-(thiophene-2-carbonyl)-pyrazolo[1,5-a]pyrimidine-7-yl]phenyl} S-acetamide), **2c** (*N*-(4-fluoro-butyl)-*N*-{3-[3-(thiophene-2-carbonyl)-pyrazolo[1,5-a]pyrimidine-7-yl]phenyl}-acetamide), **3** (7-{3-[(2-fluoroethyl)(-methyl)-amino]-phenyl}-pyrazolo[1,5-a]pyrimidine-3-yl](-thiophen-2-yl)-methanone), and **4** (*N*-{3-[3-(4-fluoro-benzoyl)-pyrazolo[1,5-a]pyrimidine-7-yl]phenyl}-*N*-methyl-acetamide) will be published elsewhere (Hoepping et al., in press). Zaleplon was a kind gift from H. Lundbeck A/S, Copenhagen, Denmark. The structures of all investigated compounds are given in Fig. 1.

2.2. Radioligand binding assays

[*N*-methyl-^{3}H]flunitrazepam (A_{spec}: 3600 GBq/mmol) was obtained from Amersham, GE Healthcare, and zolpidem from TOCRIS, Biotrend, Germany. If not otherwise stated, all other solvents and chemicals were provided by Perkin Elmer Life Sciences, Netherlands, Sigma, Germany, and Merck, Germany.

All procedures involving animals were carried out following national regulations for animal research and in accordance with the Declaration of Helsinki. Newborn (postnatal day 1; P1) and 8-week old (adult) female Sprague–Dawley rats were anaesthetized with CO_2/O_2, decapitated, and the brains were dissected rapidly on ice. To obtain tissue membranes, whole brains of rats decapitated at P1 and cerebella of adult rats were collected and homogenised in ice-cold buffer (50 mM Tris–HCl, pH 7.4 at