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In vitro toxicity of the galanin receptor 3 antagonist SNAP 37889

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ABSTRACT

Galanin and its receptors (GAL_1, GAL_2, GAL_3) modulate a range of neuronal, immune and vascular activities. *In vivo* administration of SNAP 37889 (1-phenyl-3-[[3-(trifluoromethyl)phenyl]imino]-1H-indol-2-one), a potent small non-peptidergic antagonist of GAL_3, was reported to reduce anxiety- and depression-related behavior, ethanol consumption, and antagonizes the effect of galanin on plasma extravasation in rodent models. Accordingly, SNAP 37889 has been proposed as a potential therapeutic agent to treat anxiety and depression disorders. Therefore, we evaluated the toxicity of SNAP 37889 to different cell types.

Our experiments revealed that SNAP 37889 (≥10 μM) induced apoptosis in epithelial (HMCB) and microglial (BV-2) cell lines expressing endogenous GAL_3, in peripheral blood mononuclear cells and promyelocytic leukemia cells (HL-60) expressing GAL_2, and in a neuronal cell line (SH-SY5Y) lacking galanin receptor expression altogether. In conclusion, SNAP 37889 is toxic to a variety of cell types independent of GAL_3 expression. We caution that the clinical use of SNAP 37889 at doses that might be used to treat anxiety- or depression- related diseases could have unexpected non-galanin receptor-mediated toxicity, especially on immune cells.

1. Introduction

Galanin shows widespread distribution in the central nervous system (CNS) and peripheral nervous system (PNS) as well as in the immune, endocrine and endothelial vascular systems (Lang et al., 2007). Galanin controls diverse physiological processes such as arousal/sleep, energy and osmotic homeostasis, reproduction, nociception and cognition. Furthermore, galanin has been correlated with pathological processes of the nervous system, including nerve injury (Hokfelt et al., 1987), Alzheimer's disease (Chan-Palay, 1988) and epileptic seizures (Mazarati et al., 1998). In the periphery, galanin plays a role in inflammatory disorders (Lang and Kofler, 2011).

Galanin's functions are mediated by three galanin receptors (GAL_1, GAL_2 and GAL_3) with peculiar distributions in the CNS, PNS, and in other tissues of the body (Lang et al., 2007). The three receptors are members of the G-protein coupled receptor (GPCR) superfamily and show differences in functional coupling, which explains disparate physiological functions of galanin in the different parts of the body. GAL_1 mRNA is found in brain regions mainly involved in appetite control, addiction, nociception and memory, in both rodents and humans (Ash and Djouma, 2011). GAL_2 is widely expressed throughout the CNS and PNS and has crucial roles in regulation of cardiovascular activity, neuroendocrine function, reproduction, digestion, and bone remodeling (Ash and Djouma, 2011). Studies on GAL_3 distribution are limited but the receptor is known to be expressed in areas of the brain involved in feeding, reward, memory and emotions (Ash and Djouma, 2011). Therapeutic modulation of galanin functions in addiction and mood disorders is considered a possible treatment for these diseases (Ash and Djouma, 2011). Therefore, attempts are ongoing to develop chimeric peptides or non-peptidergic compounds to specifically stimulate or inhibit galanin (GAL) receptors.

Swanson et al. (2005) developed two non-peptidergic GAL_3 selective antagonists, 1-phenyl-3-[[3-(trifluoromethyl)phenyl]imino]-1H-indol-2-one (SNAP 37889) and (1-(3-(2-pyrrolidin-1-ylethoxy)phenyl)-3-(3-(trifluoromethyl)phenyl)iminoindol-2-one (SNAP 398299). The compounds were described as having high selectivity for GAL_3 and low or no selectivity for GAL_1 and GAL_2 (Swanson et al., 2005). SNAP 37889 administrated intraperitoneal (i.p.) or orally was reported to significantly decrease anxiety and depression in rodents (Swanson et al., 2005). In addition, i.p. administration of SNAP 37889 has been shown to reduce

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Table 1
Relative expression levels of galanin receptors to the housekeeping gene RPL27 (human) or HPRT (murine).

	GAL_1	GAL_2	GAL_3
HMCB[a]	+++	−	+
SH-SY5Y	−	−	−
HL-60	−	+	−
PBMCs	−	+	−
BV-2[b]	−	+	++

+++: ΔCt below 5; ++: ΔCt between 5 and 10; +: ΔCt higher than 10; −: no expression.

[a] Expression without quantification reported by Habert-Ortoli et al. (1994) and Lang et al. (2001).

[b] Expression without quantification reported by Su et al. (2003).

alcohol consumption of rats (Ash et al., 2011, 2014). Furthermore, SNAP 37889 reduced inflammation in a model of acute pancreatitis and neurogenic skin inflammation (Barreto et al., 2011; Schmidhuber et al., 2009; Swanson et al., 2005). As SNAP 37889 could have potential as a therapeutic drug for several diseases, the present study evaluated possible toxic effects of SNAP 37889 on several cell types with differing patterns of endogenous expression of GAL receptors.

2. Materials and methods

2.1. Materials

Cell culture media and supplements were obtained from Sigma-Aldrich (St. Louis, MO, USA) unless stated otherwise. Tissue culture flasks and tubes were purchased from Greiner Bio-One (Frickenhausen, Germany). CytoOne 12/24-well plates were obtained from STARLAB (Hamburg, Germany). SNAP 37889 was obtained from Key Organics (Camelford, UK) and dissolved in 5% dimethylsulfoxide (DMSO), 1% hydroxypropylmethyl-beta-cellulose (HPM-β-C) v/v (vehicle) at a concentration of 1 mM and stored at −80 °C.

2.2. Cell culture

SH-SY5Y cells (human neural crest-derived neuroblastoma cells; ATCC CRL-2266) were maintained in 1:1 (v/v) minimal essential medium (MEM)/Ham's F-12 medium containing 10% Fetal bovine serum (FBS) (GE Healthcare Bio-Science AB, Uppsala, Sweden), 1× MEM non-essential amino acid solution, 2 mM GlutaMAX (Gibco, Life Technologies GmbH, Darmstadt, Germany) and 1× penicillin–streptomycin–fungizone (Pen/Strep/Fung) mixture (Lonza, Basel, Switzerland).

HMCB cells (human melanoma cells Bowes; ATCC CRL-9607) were maintained in MEM containing 10% FBS, 2 mM GlutaMAX, 1 mM sodium pyruvate, 10 mM HEPES and 1× Pen/Strep/Fung mixture.

HL-60 cells (human promyelocytic leukemia cells; ATCC CCL-240) were maintained in Iscove's modified Dulbecco's medium (IMDM) containing 10% FBS and 1× Pen/Strep/Fung mixture.

BV-2 cells (murine microglial cell line; (Blasi et al., 1990)) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 2200 mg glucose/L, 10% FBS (Gibco, Life Technologies GmbH, Darmstadt, Germany) and 1× Pen/Strep/Fung mixture at 37 °C in a humidified atmosphere of 5% CO_2.

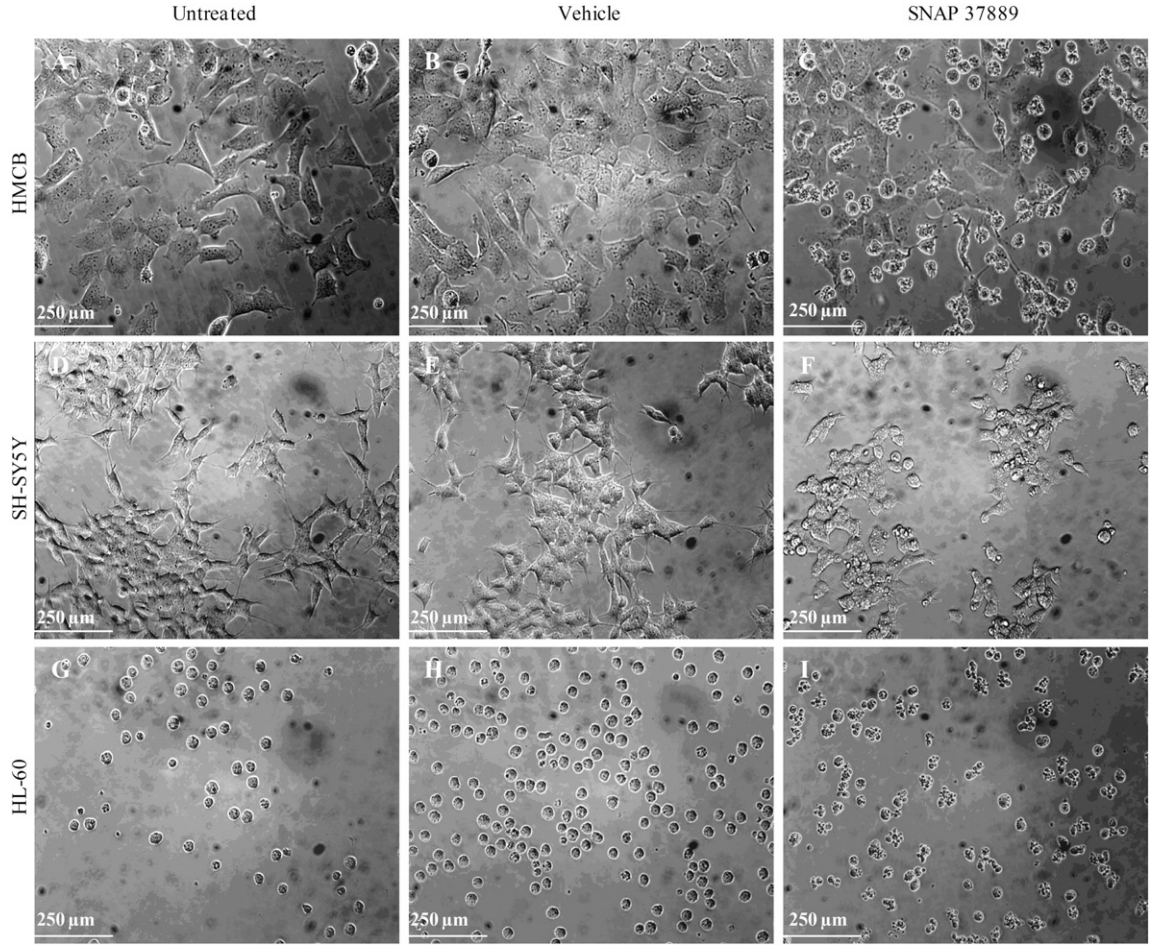

Fig. 1. Morphology of HMCB (A–C), SH-SY5Y (D–F) and HL-60 (G–I) cells. The cell lines were left untreated (A; D; G), treated with vehicle alone (B; E; H) or treated with 10 µM SNAP 37889 (C; F; I) for 4 h.