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Anxiolytic properties of a 2-phenylindolglyoxylamide TSPO ligand: Stimulation of *in vitro* neurosteroid production affecting $GABA_A$ receptor activity

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Summary A number of neurosteroids have been demonstrated to exert anxiolytic properties by means of a positive modulation of inhibitory GABAergic neurotransmission. The observation that neurosteroid synthesis can be pharmacologically regulated by ligands to the mitochondrial translocator protein (TSPO) has prompted the search for new, more selective TSPO ligands able to stimulate steroidogenesis with great efficacy. In the present study, the potential anxiolytic activity of a selective TSPO ligand, *N*,*N*-di-*n*-propyl-2-(4-methylphenyl)indol-3-ylglyoxylamide (MPIGA), was tested by means of the elevated plus maze paradigm. Moreover, the *in vitro* effects on synaptoneurosomal $GABA_A$ receptor ($GABA_AR$) activity exerted by the conditioned salt medium from MPIGA-treated ADF human glial cells were investigated. MPIGA (30 mg/kg) was found to affect rats' performance in the elevated plus maze test significantly, leading to an increase in both entries and time spent in the open arms. This same dose of MPIGA had no effect on rats' spontaneous exploratory activity. The conditioned salt medium from MPIGA-treated ADF cells potentiated the $^{36}Cl^-$ uptake into cerebral cortical synaptoneurosomes. The exposure of ADF cells to MPIGA stimulated the production of pregnelonone derivatives including allopregnanolone, one of the major positive $GABA_AR$ allosteric modulator. In conclusion, the TSPO ligand MPIGA is a

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promising anxiolytic drug. The mechanism of action by which MPIGA exerts its anxiolytic activity was identified in the stimulation of endogenous neurosteroid production, which in turn determined a positive modulation of $GABA_AR$ activity, thus opening the way to the potential use of this TSPO ligand in anxiety and depressive disorders.

1. Introduction

Anxiety disorders are the most common and frequent mental disorders, affecting a significant percentage of the world's population (8–13%). The level of specific neurosteroids has been shown to be altered in anxiety and major depression disorders, emphasizing the involvement of these endogenous molecules in the pathophysiology of such psychiatric disorders. Notably, in addition to their well-documented effect as regulators of gene expression, a number of neurosteroids are able to alter neuronal excitability by interacting with membrane-bound neuro-transmitter receptors (Baulieu, 1998). Neurosteroids, such as pregnenolone and the progesterone metabolite allopregnanolone, rapidly inhibit CNS excitability as a result of a stereoselective and high affinity interaction with type A receptors for GABA ($GABA_A$), the major inhibitory neuro-transmitter in the brain (Hiemke et al., 1991; Lambert et al., 2003). The binding of these neuroactive steroids to $GABA_A$ receptors ($GABA_ARs$) results in potentiation of GABA-induced Cl^- currents (Hosie et al., 2007), which may account for their anxiolytic, anticonvulsant, hypnotic and anaesthetic effects (Strous et al., 2006). For some of these so-called "neuroactive steroids", considerable evidence exists for their *de novo* synthesis from cholesterol in the brain, mainly in glial cells, independently of peripheral endocrine sources (Baulieu, 1998). Brain concentrations of neurosteroids can be affected by selective activation of the mitochondrial translocator protein (TSPO), expressed at a high level in glial cells. TSPO is a key element of the mitochondrial import machinery responsible for supplying the substrate cholesterol to the first steroidogenic enzyme (P450ssc), which transforms cholesterol into pregnenolone, the precursor of all neurosteroids (Papadopoulos et al., 2006; Rone et al., 2009). It has been widely demonstrated that TSPO ligands may stimulate pregnenolone formation in steroidogenic cell models (Krueger and Papadopoulos, 1990; Guarneri et al., 1992; Romeo et al., 1992; Cascio et al., 1999; Primofiore et al., 2004; Selleri et al., 2005). In addition, TSPO ligands have been found to increase pregnenolone levels *in vivo* in the rat forebrain and hippocampus, and, consequently, they are able to elicit antineophobic, anticonflict and anxiolytic actions (Korneyev et al., 1993; Costa et al., 1994; Reddy and Kulkarni, 1996; Okuyama et al., 1999; Serra et al., 1999; Bitran et al., 2000; Trapani et al., 2005; Verleye et al., 2005; Da Settimo et al., 2008; Taliani et al., 2009).

The observation that central synthesis of neuroactive steroids can be pharmacologically modulated has prompted a search for new, selective TSPO ligands that are able to stimulate steroidogenesis with great efficacy. *N*,*N*-Dialkyl-2-phenylindol-3-ylglyoxylamides have recently been reported as a novel class of TSPO ligands possessing high affinity and selectivity. Most of these compounds showed a greater ability to stimulate pregnenolone biosynthesis in rat C6 glioma cells than that of classic TSPO ligands (Primofiore et al., 2004; Da Settimo et al., 2008). In particular, the *N*,*N*-di-*n*-propyl-2-(4-methylphenyl)indol-3-ylglyoxylamide (MPIGA) showed all the above-described characteristics (K_i = 5.5 nM; increase in pregnenolone production 148% *vs* control), and presented theoretically calculated physicochemical properties basically fulfilling the requirements for an adequate distribution into the CNS. Based on this evidence, the compound MPIGA was evaluated for its potential anxiolytic activity in the present work, by means of the elevated plus maze paradigm, after *in vivo* administration in rats. In order to clarify the compound mechanism at the cellular level, an *in vitro* cellular system resembling the glial–neuron interaction was developed: the human glial cell line was treated with MPIGA, and the conditioned cell medium was analyzed for its ability (1) to modulate the GABA-evoked radioisotopic chloride ($^{36}Cl^-$) uptake into synaptoneurosomes, and (2) to affect agonist binding to $GABA_AR$ in membrane homogenate. Furthermore, the concentrations of a number of neurosteroids that regulate the $GABA_AR$ activity were evaluated in the conditioned medium from MPIGA-treated ADF cells.

2. Methods and materials

2.1. Theoretical calculation of MPIGA physicochemical properties

MPIGA drugability was assessed by means of C Log *P*, ASA and TPSA calculations. Analyses were performed using the Marvin Sketch and Calculator Plugins available on the web-site: http://www.chemaxon.com/demosite/marvin/index.html.

2.2. Evaluation of anxiolytic effects following MPIGA administration in rats

The elevated plus maze (EPM) paradigm was used to evaluate the anxiolytic effects of the TSPO ligand MPIGA (Dawson and Tricklebank, 1995; Rodgers and Dalvi, 1997). Briefly, the apparatus was made of white PVC and consisted of two opposite open arms (length 50 cm, width 10 cm) and two opposite closed arms (length 50 cm, width 10 cm), the latter enclosed by 40 cm high walls along their length. The four arms converged to a central square (10 cm × 10 cm), thus reproducing the shape of a plus sign. The apparatus was elevated 50 cm from the floor. Rats having no prior experience of the elevated plus maze were placed in the central square, and were left free to explore the whole apparatus for a single 5 min test session. The experiments were performed under an illumination of 40 lux, which was uniform in both the open and closed arms of the apparatus. Rat performance was videotaped, and percentages of arm entries as well as of time spent in open and closed arms were calculated with respect to the total number of entries and to the total amount of time spent in the arms, respectively. A rat was considered inside a specific arm when