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Neuroprotective effects of steroid analogues on P19-N neurons

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Abstract

Naturally occurring neurosteroids are potent allosteric modulators of γ-aminobutyric acid$_A$ receptor and through augmentation of γ-aminobutyric acid$_A$ receptor function, can protect neuronal cells against *N*-methyl-D-aspartate receptor over-activation, ischemia and traumatic brain injury. In this study, mouse P19 cells were induced to differentiate into post-mitotic neurons and were subjected to excitotoxicity in the presence of *N*-methyl-D-aspartate. Novel synthetic analogues of the endogenous neurosteroids allopregnanolone and dehydroepiandrostrone, inhibited excitotoxic cell death of P19-N neurons, by directly maintaining the activation of PKB/Akt kinase and interfering with the intrinsic mitochondrial apoptotic pathway, preserving cytochrome *c* in the mitochondria and Bax in the cytoplasm. The efficiency and the potency of these neurosteroids were similar to those of allopregnanolone and dehydroepiandrostrone. Their effects were γ-aminobutyric acid$_A$ receptor mediated, since they were abolished in the presence of bicuculline, an antagonist of receptor's function. In addition, the synthetic compounds retained the ability to alter γ-aminobutyric acid$_A$ receptor subunit gene expression, but their effects on transcriptional activity were less pronounced than those of allopregnanolone and dehydroepiandrostrone. These results suggest that synthetic neurosteroids may serve as potent, membrane acting, neuroprotectants against *N*-methyl-D-aspartate receptor neurotoxicity on neuronal cells.

Keywords: Synthetic neurosteroids; NMDA cytotoxicity; GABA$_A$ receptor; Cytochrome *c*; Neuroprotection

1. Introduction

There is increasing evidence that growth and protection of neuronal cells is promoted by steroid hormones like progesterone and its metabolites (Baulieu and Robel, 1990; Baulieu, 1997) or estrogens, like 17β-estradiol (Keller et al., 2005; Tunez et al., 2006). The nervous system is also a steroidogenic tissue, since it produces steroids (designated as neurosteroids) that have a paracrine or autocrine effect on neurons and glial cells (Majewska et al., 1986; Baulieu and Robel, 1990; Compagnone and Mellon, 2000). Mechanisms by which neuroactive steroids, like allopregnanolone (allo), dehydroepiandrosterone (DHEA) and its sulfate ester (DHEAS), act in the central nervous system (CNS), include both genomic actions, mediated by nuclear steroid hormone receptors and non-genomic actions, mediated by neurotransmitter-gated ionotropic receptors, such as the γ-aminobutyric acid type A (GABA$_A$R) and the *N*-methyl-D-aspartate (NMDAR) receptors, or through G protein-coupled receptors (Rupprecht et al., 1996; Rupprecht and Holsboer, 1999; Belelli and Lambert, 2005). The production rate and the levels of neurosteroids decline rapidly with age, increasing the vulnerability of neurons to neurodegenerative processes (Baulieu, 1997; Weill-Engerer et al., 2002). It seems therefore that these steroids are able to protect CNS neurons against the neurotoxic action of *N*-methyl-D-aspartate (NMDA) (Frank and Sagratella, 2000; Lockhart et al., 2002) and to decrease cell death and cognitive deficits after traumatic brain injury (Djebaili et al., 2004, 2005). In several cases, the neuroprotective effects of neurosteroids have been shown to be mediated by activation of the phosphatidylinositol 3-kinase (PI3-K)/Akt cascade (Zhang et al., 2002; Charalampopoulos et al., 2004). The serine-threonine protein kinase Akt/PKB is implicated in survival signaling in many cell types, including neurons (Dudek et al., 1997; Kulik and Weber, 1998), where activation of Akt by growth factor stimulation has been shown to enhance neuronal survival through inhibition of apoptosis (Downward, 1998; Datta et al., 1999, 2002; Brazil et al., 2002).

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However, naturally occurring neurosteroids have therapeutic limitations because they are rapidly metabolized, presumably by conjugation of the 3α-hydroxyl or oxidation to the corresponding ketones (Craig and Deason, 1968; Rupprecht, 1997; Beekman et al., 1998). Consequently, synthetic analogues of endogenous neurosteroids may be clinically useful neuroprotectants, anaesthetics and anticonvulsants, through augmentation of $GABA_A$ergic transmission and blockage of NMDAR-mediated transmission (Majewska et al., 1986; Macdonald and Greenfield, 1997; Gasior et al., 1999; Zorumski et al., 2000; Hamilton, 2002). Recently, we have reported on the in vitro binding affinity for $GABA_AR$ of synthetic allopregnanolone derivatives substituted by conformationally constrained 17β side chains. The most potent were the propadienyl pregnanediol TC 285 and the alkynyl pregnanediol TC 286, exhibiting K_s values of 2.8 ± 1.3 and 145 ± 49 nM, respectively, which are significantly lower than the K_s value of allopregnanolone (198 ± 47 nM) (Souli et al., 2005). In the present report, we have studied the neuroprotective properties of these allopregnanolone derivatives, along with the 3α-methyl substituted DHEA analogue (TC 22) (Fig. 1), against NMDA-induced excitotoxicity on P19-derived neurons and the possible implication of PI3K/Akt signaling pathway in these effects. The rationale for the synthesis of TC 22 was based on previous reports which claimed that metabolic oxidation of the 3-hydroxy group in steroid derivatives, can be blocked by the addition of another substituent at the C3 position of the steroid skeleton (Gee et al., 1995; Upasani et al., 1997).

P19 cells can be induced to differentiate into post-mitotic neuron-like cells, expressing a wide variety of neuron specific markers, as well as, a diversity of functional ion channels and receptors, including $GABA_AR$ and NMDAR (McBurney et al., 1988; Finley et al., 1996; MacPherson et al., 1997; Chistina Grobin et al., 1999). We have recently reported that allopregnanolone protects P19-N differentiated neurons from NMDA-induced apoptosis, preserving cytochrome *c* in the mitochondrion and Bax in the cytoplasm, while allo and DHEA induce the expression of α1 and β2 $GABA_AR$ subunit mRNAs (Xilouri and Papazafiri, 2006).

2. Experimental procedures

2.1. Materials

Fetal calf serum, alpha-minimal essential medium (a-MEM), penicillin–streptomycin, L-glutamine, non-essential amino acids and trypsin, were purchased from PAA laboratories (Pasching, Austria). Neurobasal medium, B-27 supplement, Trizol reagent, Superscript II Rnase H-Reverse Transcriptase, were purchased from Gibco, Invitrogen (Carlsbad, USA). Taq DNA

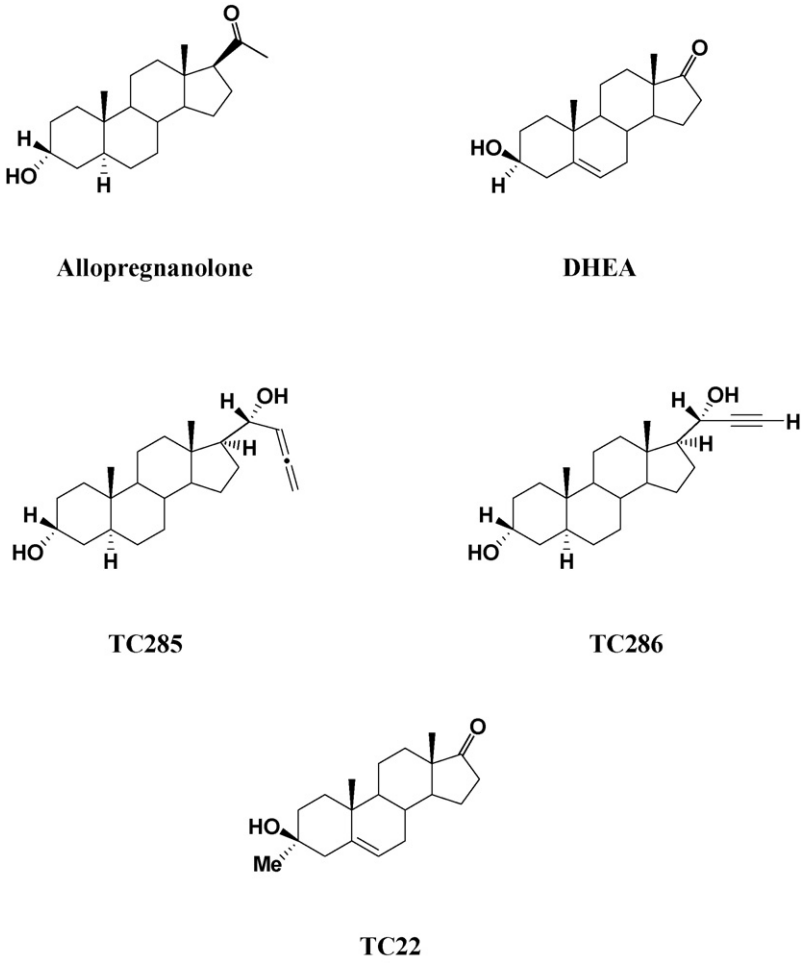

Fig. 1. Chemical structure of neurosteroid analogues.