ELSEVIER

European Journal of Pharmacology 409 (2000) R1–R2

ejp

www.elsevier.nl/locate/ejphar

Rapid communication

3α-Hydroxy-5α-pregnan-20-one exposure reduces $GABA_A$ receptor α4 subunit mRNA levels

A. Chistina Grobin*, A. Leslie Morrow

Department of Psychiatry, Pharmacology and Bowles Center for Alcohol Studies, CB #7178, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7178, USA

Received 17 October 2000; accepted 18 October 2000

Abstract

To examine the direct effects of neurosteroids on γ-aminobutyric acid type A ($GABA_A$) receptor expression, we exposed developing neuronal cells (P19) in vitro to 3α-hydroxy-5α-pregnan-20-one (3α,5α-THP, allopregnanolone). Quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) analysis revealed a concentration-dependent decrease in $GABA_A$ receptor α4 subunit mRNA expression that reversed 24 h after steroid withdrawal. These data suggest that variations in neurosteroid levels regulate the pattern of $GABA_A$ receptor subunit expression and may alter the trophic effects of GABA.

Keywords: Neurosteroid; Development; P19 cell

γ-Aminobutyric acid (GABA), acting primarily through $GABA_A$ receptors is the major inhibitory neurotransmitter in adult mammalian brain whereas in developing neurons, $GABA_A$ receptors appear to transduce a trophic signal in developing neurons (Behar et al., 1996). The pregnane steroid 3α-hydroxy-5α-pregnan-20-one (3α,5α-THP, allopregnanolone) is one of the most potent modulators of $GABA_A$ receptor function. It is present in pharmacologically relevant concentrations during neuronal development (Kellogg and Frye, 1999) and alters $GABA_A$ receptor expression and function in vivo (Smith et al., 1998a).

The goal of the present study was to determine if alterations in 3α,5α-THP levels affect $GABA_A$ receptor α4 subunit mRNA expression in developing neurons. P19 cells are embryonic teratocarcinoma cells that can differentiated in vitro into neurons and glial by treatment with retinoic acid. These cells have functional $GABA_A$ receptors and express the most common $GABA_A$ receptor subunits (Grobin et al., 1999). Similar to other neuronal culture systems, these cells are immature relative to adult mammalian brain and can be considered a model for developing GABA neurons.

P19 cells were maintained, differentiated with retinoic acid and plated as previously described (Grobin et al., 1999). Seven days after final plating, cultures were treated with varying concentrations of 3α,5α-THP (0.01–10 μM) or vehicle (20% β-cyclodextrins vol/vol) and allowed to survive for 4 days. For cultures subjected to withdrawal, steroid containing media was removed and fresh media applied. Cultures were harvested 24 h later. 3α,5α-THP was extracted from cell culture media as previously described (Janis et al., 1998). Sample extracts were subjected to radioimmuno assay (RIA) determination of 3α,5α-THP levels using a sheep polyclonal antibody. Competitive reverse transcriptase-polymerase chain reaction (RT-PCR) assay using an α4 subunit specific internal standard was conducted as previously described (Grobin et al., 1999). Data were analyzed by one-way analysis of variance followed by Dunnett's post hoc analysis.

Fig. 1 illustrates that addition of 3α,5α-THP to culture medium of developing neurons resulted in a concentration-dependent decrease in $GABA_A$ receptor α4 subunit mRNA expression. 3α,5α-THP concentrations of 0.1, 1 and 10 μM significantly lowered α4 mRNA levels. This depression was reversed 24 h after withdrawal from 3α,5α-THP. No elevation of α4 subunit mRNA levels

* Corresponding author. Tel.: +1-919-966-4977; fax: +1-919-966-9099.

E-mail address: grobinac@med.unc.edu (A.C. Grobin).

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00797-4

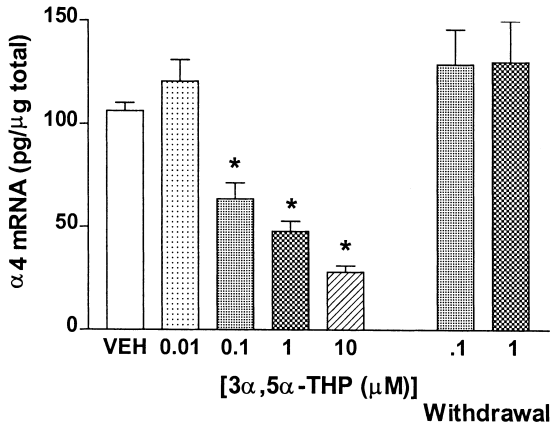

Fig. 1. 3α,5α-THP concentration alters $GABA_A$ receptor α4 subunit mRNA levels in differentiated P19 cells. Differentiated P19 cells were exposed to varying concentrations of 3α,5α-THP for 4 days. Absolute α4 subunit mRNA levels were determined by competitive, quantitative RT-PCR analysis using an α4 subunit specific cRNA standard. α4 subunit mRNA levels were significantly decreased ($^*P < 0.05$) when exposed to 0.1, 1 and 10 μM 3α,5α-THP. Withdrawal (24 h) from 3α,5α-THP reversed the effect of 3α,5α-THP on α4 mRNA levels. Data shown are the summary (mean ± S.E.M.) of 3–5 independent exposures. RIA analysis of 3α,5α-THP levels in cell culture media confirmed the concentration of added steroid and indicated that 3α,5α-THP levels in normal media and vehicle (VEH)-treated cells were negligible (below detection limits).

was observed following removal of 3α,5α-THP from culture medium.

These data demonstrate that neurosteroid induced $GABA_A$ receptor α4 subunit mRNA expression is regulated differentially depending on the model employed. 3α,5α-THP exposure alters $GABA_A$ receptor properties in vitro including decreased [^{35}S]t-butylbicyclo-phosphothionate (TBPS) binding and GABA-mediated $^{36}Cl^-$ influx, and decreases in other $GABA_A$ receptor subunit (α2, α3 and β) mRNAs (Yu et al., 1996) and increases in α4 subunit mRNA levels (Fig. 1). However, in vivo exposure to 3α,5α-THP during pregnancy and pseudopregnancy results in increased TBPS binding (Concas et al., 1998), anxiolysis (Smith et al., 1998b) and no change in $GABA_A$ α4 subunit mRNA (Concas et al., 1998). Thus, in vitro studies may not completely reflect the in vivo environment, or alternatively, other hormones may exert genomic effects during pregnancy.

Model-dependent results were also apparent during steroid withdrawal; removal of 3α,5α-THP from the culture media did not result in elevated α4 subunit mRNA levels. Artificial withdrawal of pregnane steroids has been advanced as a model for the postpartum period and shown to induce profound increases in $GABA_A$ receptor α4 subunit mRNA expression in the hippocampus (Smith et al., 1998a). Our data show α4 subunit mRNA levels during 3α,5α-THP withdrawal are indistinguishable from vehicle-treated cultures, consistent with cortical α4 subunit mRNA observed during the physiological postpartum period (Concas et al., 1998). Our data are taken from differentiated cells and may reflect developmental regulatory processes. However, the animals undergoing pseudopregnancy induction are immature (Smith et al., 1998b) and therefore it is unclear if alterations in α4 subunit mRNA reflect the developmental stage in vivo or involve multiple hormone influences.

These data provide a better understanding of the role of endogenous GABAergic neurosteroids in normal neuronal development. This work has potential ramifications for stress sensitive psychiatric disorders including schizophrenia, post-traumatic stress disorder and depression. Additionally, ascribing a neurodevelopmental function to 3α,5α-THP has implications for the acute and chronic use of other $GABA_A$ receptor modulators, most notably benzodiazepines, barbiturates and ethanol.

Acknowledgements

The authors are grateful to Margaret J. VanDoren for 3α,5α-THP level determinations in culture media. The present work was supported by the National Institute on Alcohol Abuse and Alcoholism grant AA09013.

References

Behar, T.N., Li, Y.-X., Tran, H.T., Ma, W., Dunlap, V., Scott, C., Barker, J.L., 1996. GABA stimulates chemotaxis and chemokinesis of embryonic cortical neurons via calcium-dependent mechanisms. J. Neurosci. 16, 1808–1818.

Concas, A., Mostallino, M.C., Porcu, P., Follesa, P., Barbaccia, M.L., Trabucchi, M., Purdy, R.H., Grisenti, P., Biggio, G., 1998. Role of brain allopregnanolone in the plasticity of gamma-aminobutyric acid type A receptor in rat brain during pregnancy and after delivery. Proc. Natl. Acad. Sci. U. S. A. 95, 13284–13289.

Grobin, A.C., Inglefield, J.R., Schwartz-Bloom, R.D., Devaud, L.L., Morrow, A.L., 1999. Fluorescence imaging of $GABA_A$ receptor-mediated intracellular Cl^- in P19-N cells reveals unique pharmacological properties. Brain Res. 827, 1–11.

Janis, G.C., Devaud, L.L., Mitsuyama, H., Morrow, A.L., 1998. Effects of chronic ethanol consumption and withdrawal on the neuroactive steroid 3α-hydroxy-5α-pregnan-20-one in male and female rats. Alcohol.: Clin. Exp. Res. 22, 2055–2061.

Kellogg, C.K., Frye, C.A., 1999. Endogenous levels of 5 alpha-reduced progestins and androgens in fetal vs. adult rat brains. Brain Res. Dev. Brain Res. 115, 17–24.

Smith, S.S., Gong, Q.H., Hsu, F.-C., Markowitz, R.S., Ffrench-Mullen, J.M.H., Li, X., 1998a. $GABA_A$ receptor α4 subunit suppression prevents withdrawal properties of an endogenous steroid. Nature 392, 926–930.

Smith, S.S., Gong, Q.H., Li, X., Moran, M.H., Bitran, D., Frye, C.A., Hsu, F., 1998b. Withdrawal from 3α-OH-5α-pregnan-20-one using a pseudopregnancy model alters the kinetics of hippocampal $GABA_A$-gated current and increases the $GABA_A$ receptor α4 subunit in association with increased anxiety. J. Neurosci. 18, 5275–5284.

Yu, R., Follesa, P., Ticku, M.K., 1996. Down-regulation of the GABA receptor subunits mRNA levels in mammalian cultured cortical neurons following chronic neurosteroid treatment. Brain Res. Mol. Brain Res. 41, 163–168.