Available online at www.sciencedirect.com
SCIENCE DIRECT
Neuropharmacology 50 (2006) 807–813
NEURO PHARMACOLOGY
www.elsevier.com/locate/neuropharm

Transforming growth factor-α induces sex-specific neurochemical imbalance in the stress- and memory-associated brain structures

Kyoko Koshibu [a,b,**], Pat Levitt [c,d,*]

[a] Department of Neurobiology, University of Pittsburgh, PA 15261, USA
[b] Center for Neural Basis of Cognition, University of Pittsburgh, PA 15261, USA
[c] Vanderbilt Kennedy Center for Research on Human Development, Vanderbilt University, Nashville, TN 37203, USA
[d] Department of Pharmacology, Vanderbilt University, Nashville, TN 37203, USA

Received 13 August 2005; received in revised form 30 November 2005; accepted 2 December 2005

Abstract

Transforming growth factor-α (TGFα) is a well-known regulator of many developmental processes. However, its role in adult nervous system is yet unclear. Studies have shown that TGFα can regulate stress and memory behavior in adult mice. When TGFα is reduced in *Waved-1* (*Wa-1*) mutant mice, the stress response and memory are impaired predominantly in males and only after puberty. To determine the neurochemical changes resulting from the reduced TGFα levels that could explain the reported behavioral outcomes, biogenic amine and amino acid levels were determined in the brain regions associated with stress and memory. Interestingly, sex-specific alterations in neurochemical levels were detected, including elevated noradrenaline and reduced glutamate levels in striatum of *Wa-1* males, increased noradrenaline and reduced serotonin metabolite levels in hippocampus of *Wa-1* females, reduced serotonin metabolite levels in cortex and amygdala of *Wa-1* females, and reduced noradrenaline, dopamine, serotonin, glutamate and glycine levels in hypothalamus of *Wa-1* females compared to their respective controls. Increased dopamine turnover in cortex and reduced dopamine and serotonin turnover in amygdala were observed in both male and female *Wa-1* mice. The data indicate sex-specific alterations of specific neurochemicals as a result of reduced TGFα expression, which may underlie sex-dependent stress response and memory impairment in *Wa-1* mice.

Keywords: TGFα; Limbic system; Catecholamine; Memory; Stress

Transforming growth factor-α (TGFα) is a member of the ErbB receptor tyrosine kinases. Both TGFα and its receptor, epidermal growth factor receptor, are expressed in a wide range of structures in the developing and mature central nervous system of rats and mice (Gomez-Pinilla et al., 1988; Tucker et al., 1993; Weickert and Blum, 1995; Kornblum et al., 1997). Its expression is developmentally regulated, sensitive to gonadal steroids, and increases around puberty in the female rat and primate hypothalamus (Ma et al., 1994a,b,c; Koshibu and Levitt, 2005). Many neural developmental processes are regulated by TGFα, including cell differentiation (Luetteke et al., 1993), multipotent neural progenitors and stem cell proliferation (Anchan et al., 1991; Lillien and Cepko, 1992; Reynolds et al., 1992; Santa-Olalla and Covarrubias, 1995) and fate choice (Ferri et al., 1996; Burrows et al.,

Abbreviations: A, adrenaline/epinephrine; ACTH, adrenocorticotrophic hormone; DA, dopamine; DOPAC, dihydroxyphenylacetic acid; EGFR, epidermal growth factor receptor; GABA, gamma-aminobutyric acid; GLM, generalized linear model; Glu, glutamate; Gly, Glycine; HPA, hypothalamo–pituitary–adrenal; HVA, homovanillic acid; 3-MT, 3-methoxytyramine; NA, noradrenaline/norepinephrine; PVN, paraventricular nucleus; PFC, prefrontal cortex; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; TGFα, transforming growth factor-alpha; *Wa-1*, *Waved-1*.

* Corresponding author. Vanderbilt Kennedy Center for Research on Human Development, Vanderbilt University, Box 40 Peabody, 230 Appleton Place, Nashville, TN 37203, USA (P. Levitt). ** Corresponding author. University of Zürich/ETH, Brain Research Institute, Bau 55 H38, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland. Tel.: +41 1 635 33 49; fax: +41 1 635 33 03 (K. Koshibu).

E-mail addresses: koshibu@hifo.unizh.ch (K. Koshibu), pat.levitt@vanderbilt.edu (P. Levitt).

0028-3908/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2005.12.001

1997; Eagleson et al., 1997), wound healing (Schultz et al., 1987), astrogliosis (Rabchevsky et al., 1998), and angiogenesis (Schreiber et al., 1986). TGFα is also involved in the neuronal control of female puberty onset through its interaction with astrocytes in hypothalamus (Ma et al., 1992, 1994a,b,c).

A general reduction of the TGFα expression in *Waved-1* (*Wa-1*) mutant mice (Mann et al., 1993; Berkowitz et al., 1996) results in a post-pubertal, male-predominant enlargement of the lateral ventricles accompanied by impaired stress response and fear memory (Burrows et al., 2000; Koshibu et al., 2005). However, this phenotype is not detected in the complete absence of TGFα (Burrows et al., 2000). Other studies have shown that the overexpression of TGFα results in hypersensitivity to stress in males (Hilakivi-Clarke et al., 1992), but hyposensitivity to stress in females (Hilakivi-Clarke et al., 1993). These results indicate that TGFα can regulate sex-dependent stress response and memory processes in adult animals.

Our initial investigation to delineate the causes of impaired stress response and memory in *Wa-1* mice revealed no differences in the baseline or stressed levels of plasma corticosterone in male or female *Wa-1* mice compared to their respective controls (Koshibu et al., 2005). Interestingly, the plasma corticosterone is unaltered also in TGFα overexpressing transgenic mice (Hilakivi-Clarke et al., 1992), indicating that TGFα does not affect adrenal cortical function, which secretes corticosterone in response to hypothalamic–pituitary axis (reviewed in Pignatelli et al., 1998). When plasma noradrenaline (NA) and adrenaline (A) were measured, female *Wa-1* showed lower plasma NA and A levels than control females. In contrast, male *Wa-1* mice had higher plasma A concentration than control males (Koshibu et al., 2005). These studies suggest that TGFα may regulate sex-specific stress response through adrenal medullar-dependent system, which is responsible for NA, A, and dopamine secretions (reviewed in Axelrod and Reisine, 1984). Because stress can improve or hinder memory depending on the magnitude of stress and the types of memory tested in humans (Kirschbaum et al., 1996; Buchanan and Lovallo, 2001) and rodents (Roozendaal and McGaugh, 1996; Sandi et al., 1997; Shors and Servatius, 1997; de Quervain et al., 1998) in a sex-dependent manner, the altered stress-induced sympatho-adrenal-medullary system response may cause memory impairment. However, the direct link between the plasma NA and A alterations and behavioral impairments was unclear.

Thus, to determine the mechanisms by which TGFα may regulate stress response and memory in adult animals, neurochemical analyses were conducted in brain regions associated with stress and memory, including cortex, striatum, hypothalamus, hippocampus, amygdala, and locus coeruleus, in male and female, control and *Wa-1* mice. The levels of selective amino acids were determined along with the biogenic amine levels to delineate possible alternations in overall neurotransmitter levels, which may suggest general inhibitory or excitatory state of the region and resulting stimulatory or suppressive effect on catecholamine releases. Other amino acids, such as tyrosine, are catecholamine precursors and thus, were included in the analysis to assess the possible changes in kinetics of neurochemical changes. We found amino acid and biogenic amine alterations in *Wa-1* that are specific to sex and brain regions. The possible link between the neurochemical and behavioral findings due to altered TGFα level in the brain is discussed.

1. Materials and methods

1.1. Animals

Male and female, control and *Wa-1* C57Bl/6^{J} adult mice (3–4 months old) were used. Heterozygous *Wa-1* C57Bl/6^{J} mating pairs were purchased from Jackson Laboratory (Bar Harbor, ME) to establish the colony. The genotyping protocol has been described in Koshibu et al. (2005). The control group included wildtype and heterozygous mice, because the latter did not show any difference from the wildtype. Although the estrous phase was monitored by recording vaginal color and opening daily in all post-pubertal females, none of the measurements were affected by the estrous cycle (data not shown).

All mice were group-housed and maintained in a central facility at University of Pittsburgh School of Medicine in a controlled environment with a 12-h light/dark cycle and free access to food and water. Experiments were performed in accordance with the guidelines provided by University of Pittsburgh Institutional Animal Care and Use Committees (IACUC) and the National Institute of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering and to reduce the number of animals used. Because correlation(s) between behavioral and anatomical results and neurochemical measurements were sought, we could not use in vitro techniques as a substitute.

1.2. Tissue extraction

Brains were extracted after cervical dislocation and decapitation and frozen in liquid nitrogen. Cortex, striatum, hippocampus, amygdala, hypothalamus, and locus coeruleus tissue punches were collected from frozen brain sections of control and *Wa-1*, male and female adult mice. Thirteen control males, 9 *Wa-1* males, 14 control females, and 15 *Wa-1* female adult mouse brain samples were obtained per area. Samples were treated as described in Cransac et al. (1996). In brief, tissues were sonicated in 250–750 μl 0.1 M TCA (0.01 M sodium acetate, 0.1 μM EDTA, and 10.5% methanol at pH 3.8), and then centrifuged at 10,000 × *g* for 20 min. The supernatant and pellets were stored separately at −80 °C. The pellets were reconstituted in the same volume of 0.5 N HCl as used for the tissue homogenization and total protein concentration was determined using BCA Protein Assay Kit (Pierce Chemical Company, Rockford, IL). The supernatants were analyzed for biogenic amines and amino acids. Both protein concentration and HPLC analyses were conducted by the Neurochemistry Core Laboratory at the John F. Kennedy Center for Research on Human Development, Nashville, TN.

1.3. HPLC for biogenic amines

Biogenic amines were determined by a specific HPLC assay utilizing an Antec Decade (oxidation: 0.7) electrochemical detector and following the method by Lindsey et al. (1998). Briefly, supernatants were thawed, spun for 20 min, then 50–150 μl were injected into a Waters Nova-Pak C18 HPLC column using a Water 717+ autosampler. Biogenic amines were eluted with a mobile phase consisting of 89.5% 0.1 M TCA, 0.01 M sodium acetate, 0.1 μM EDTA and 10.5% methanol (pH 3.8). Solvent was delivered at 0.7 ml/min using a Waters 515 HPLC pump. In some cases, the supernatant was purified and concentrated by adsorption of the amines (adrenalin, noradrenalin, and dopamine) to solid alumina (Al_2O_3) to improve the accuracy of the measurements (Mefford, 1985). Dihydroxybenzylamine (internal standard), Tris buffer (pH 8.5), 100 mg Al_2O_3, and 100–200 μl of supernatant were mixed for 15 min then the buffer was aspirated. The Al_2O_3 was washed with distilled water, water was aspirated, and the amines were desorbed from the Al_2O_3