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Alterations in Brain Monoamines and $GABA_A$ Receptors in Transgenic Mice Overexpressing TGFα

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TGFα Monoamines DA transporter 5-HT_{1A} receptors 5-HT_2 receptors $GABA_A$ Transgenic mice

THE FUNCTIONAL significance of the presence of transforming growth factor α (TGFα) in the brain (37) remains largely unknown. We have investigated the behaviors of transgenic MT42 CD-1 mice that overexpress TGFα gene in multiple tissues, including the brain (32). Male transgenic TGFα mice, compared with nontransgenic CD-1 mice, are highly aggressive and exhibit increased locomotor activity in an open field and lengthened immobility in the swim-test model of

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depressive behavior, and consume more alcohol (23,25,29). The female TGFα mice, on the other hand, differ from the nontransgenic CD-1 female mice by showing less immobility in the swim test and less aggressive behavior (23,24). Our findings thus suggest that overexpression of TGFα produces gender-specific behavioral alterations (23). In particular, the male TGFα mice exhibit feminization of sexually dimorphic nonreproductive behaviors (23).

Besides behavioral alterations, overexpression of TGFα causes many other changes in MT42 transgenic mice. Both male and female TGFα mice show elevated plasma levels of 17 β-estradiol (E_2) and reduced natural killer cell activity (24,25). It is likely that the elevated plasma levels of E_2 are responsible for the alterations in various behaviors in the transgenic mice, because gonadal hormones play an important role in sexual differentiation of nonreproductive behaviors (12,43). However, it is also possible that overexpression of TGFα and/or estrogen influences neurotransmission in a gender-specific manner to induce behavioral changes. Estrogen modulates catecholamine synthesis and metabolism (8,14,47, 63). Further, estrogen alters serotonin (5-HT) content, turnover, and 5-HT receptor binding site density in some brain regions (3,16). Steroids also influence GABAergic receptors (19).

Little is known about the interaction between TGFα and neurotransmitters. Alexi and co-workers (1) reported that TGFα stimulates the uptake of dopamine (DA) in fetal dopaminergic neurons. Our behavioral results in the TGFα mice (24,25,27,28) suggest that monoaminergic and/or GABAergic transmission may be altered. Monoamines and GABA are known to participate in the control of aggressive and depressive behavior, locomotor activity, and voluntary alcohol consumption (4,13,15,18,22,44,45). The response to alcohol is also altered in the TGFα mice (27), which suggests that their GABAergic transmission may be altered. The present study investigated the brain concentrations of norepinephrine (NE), DA, and 5-HT, their metabolites, and [^{3}H]GBR 12935-labeled DA transport binding in female and male transgenic TGFα mice. Furthermore, the levels of serotonin receptors 5-HT_1 and 5-HT_2 and indicators of function of $GABA_A$ receptors were determined in the male brain.

METHODS

Subjects

Male mice of the CD-1 background were made transgenic for human growth factor TGFα and were provided by Dr. Glenn Merlino (National Cancer Institute, Frederick, MD) (32). Human TGFα binds to mouse EGF receptors with an equivalent affinity and induces similar biologic responses as mouse TGFα (6). A detailed description of the procedures to make the transgenic mice is available in the work of Jhappan et al.(32).

Nontransgenic male CD-1 mice, matched for age and housing conditions with transgenic mice, were used as controls. CD-1 mice were purchased from NCI (Frederick, MD). The purchased animals arrived at the laboratory at the age of 2–6 weeks. The mice were maintained on a 12 L : 12 D cycle and allowed ad lib access to food and water. The animals were kept in the laboratory for at least 6 weeks before they were sacrificed.

Tissue Preparation

The brains of TGFα and nontransgenic control mice were removed after cervical dislocation, dissected into several areas, and rapidly frozen in dry ice. Frozen tissues were stored at −70°C before the preparation of samples.

Monoamine Concentrations

The frontal cortex, hypothalamus, and brain stem of five male and five female TGFα and five male and five female nontransgenic mice were used. The brains were assayed for NE; DA; 5-HT; the DA metabolites, homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC); and the 5-HT metabolite, 5-hydroxyindoleacitic acid (5-HIAA). Concentrations of monoamines and metabolites were quantified using high performance liquid chromatography (HPLC) with electrochemical detection (at a potential of 0.85 V). The system consisted of a Merck-Hitachi 655A-12 Liquid Chromatograph pump (Hitachi Ltd., Tokyo, Japan), L-5000 LC-Controller, and ESA model 5100 Coulochem electrochemical detector system with a model 5020 guard cell followed by a model 5011 dual electrode analytical cell (ESA Inc., Bedford, MA), and a Merck-Hitachi Chromato-Integrator. The separation of sample components was done with a Hibar (E. Merck, Darmstadt, Germany) stainless-steel column (4 mm ID × 250 mm) packed with C18 reversed-phase LiChrospher (5 μm). The mobile phase contained 70% buffer (0.02 M trichloroacetic acid, 0.075 M sodium phosphate, 1.5 μM EDTA, and 1.5 mM lauryl sulphate), 20% acetonitrile, and 10% methanol; pH was 3.1. The flow rate was 1 ml/min. The detector potential at the analytical cell was set at +0.4 V. For the determination of monoamines the tissue samples were homogenized in ice-cold 0.1 M perchloric acid (PCA) containing 3,4-dihydroxybenzylamine hydrobromide as an internal standard. After centrifugation (4000 × *g* for 30 min), the supernatant was filtered through a 0.22-μm filter and injected into the chromatograph. The chemicals used as standards were obtained from Sigma (St Louis, MO), dissolved in 0.1 M PCA and diluted daily to the concentration used in the runs. The detection limit was 0.2–1 ng/ml.

Dopamine Transport

We used the frontal cortex and cerebellum obtained from five male and five female transgenic TGFα mice, and from five male and five female nontransgenic mice. These tissues were homogenized in 30 vol./wt. of ice-cold assay buffer, 50 mM Tris-HCl, and 5 mM KCl plus 24 mM NaCl (pH 7.9) at 4°C, with a Brinkman PT-10 Polytron at setting 6 for 10 s, and centrifuged at 50,000 × *g* for 10 min. The washing was repeated twice and the final pellet was resuspended in 25 vol of assay buffer.

The [^{3}H]GBR 12935 binding reaction was carried out according to the method of Hitri et al. (30). In the initial saturation experiments, increasing concentrations of [^{3}H]GBR 12935 (1–10 nM) were incubated with aliquotes of homogenate corresponding to 8 mg of tissue per assay tube, obtained on pooled tissues from six mice. Nonspecific binding was defined as excess over blanks that contained 1 μM GBR-12909. In a subsequent experiment each mouse cortex and cerebellum was assayed with a single concentration of [^{3}H]GBR (10 nM). The incubation was carried out in a final volume of 1 ml, at 4°C for 60 min, and the binding reaction was stopped by rapid ultrafiltration over Whatman GF/B filters. The filters were rinsed with 2 × 5 ml of ice-cold assay buffer, and the retained radioactivity was measured by conventional scintillation counting.

5-HT Receptor Assays

The frontal cortex and hippocampus of nine male TGFα and seven male nontransgenic mice were used. 5-HT$_{1A}$ receptors were assayed using [^{3}H]DPAT (135 Ci/mMol; Du Pont New England Nuclear Products, Boston, MA). 5-HT$_2$ receptors were assayed using [^{3}H]ketanserin (61 Ci/mmol; New England Nuclear Products). Tissues were suspended in 50 mM Tris-HCl buffer (pH 7.7 at 25°C), homogenized with a Brinkman Polytron (setting 6 at 10 s) and centrifuged at 40,000 × *g* for 10 min. The pellet was resuspended in fresh buffer, centrifuged, and then resuspended again in fresh buffer. For 5-HT$_2$ receptors, aliquots of this homogenate equivalent to 5 mg of tissue (approximately 0.27 mg protein) were incubated with [^{3}H] ketanserin (1 nM) at 37°C for 15 min in a final volume of 1 ml. The assays were carried out in sextuplicates, with half of the tubes containing 2 μM cinanserin to assess the nonspecific binding for 5-HT$_2$ receptors.

The remaining homogenate was preincubated at 37°C for 10 min and then centrifuged at 40,000 × *g*. The pellet was resuspended in 50 mM TRIS-HCl buffer containing 0.55 mM ascorbic acid and 10 μM pargyline. Aliquots of this homogenate equivalent to 3 mg tissue (approximately 0.17 mg protein) were incubated with [^{3}H] 8-OH-DPAT (2 nM) for 30 min at 25°C. The assays were carried out in sextuplicate, with half of the tubes containing 10 μM 5-HT to assess the nonspecific binding for 5-HT$_{1A}$ receptors.

At the end of the incubations, the samples were filtered through Whatman GF/F filters. The filters were washed three times with 4 ml cold buffer, transferred to vials, and counted by scintillation fluid spectrometry. Specific binding was defined as the difference between total and nonspecific binding, and was approximately 55% (cortex) and 70% (hippocampus) for [^{3}H]8OH-DPAT, and approximately 70% (cortex) for [^{3}H]ketanserin.

Binding to the GABA$_A$ Receptor

Forebrain hemispheres and cerebella of six male TGFα and six nontransgenic CD-1 mice were thawed, homogenized, in 50 vol ice-cold 50 mM Tris-citrate buffer (pH 7.4 at 25°C), and centrifuged at 20,000 × *g* for 20 min. The pellets were resuspended to give final suspensions with a protein content of 1 mg/ml, determined with the Bio-Rad assay using bovine serum albumin as standard. The suspensions were either used directly in [^{35}S]TBPS (88.4 Ci/mmol; New England Nuclear) binding or frozen at −80°C to be used in [^{3}H]flunitrazepam (84 Ci/mmol; Amersham) and [^{3}H]Ro 15-4513 (24.1 Ci/mmol; New England Nuclear) binding after one centrifugation–resuspension cycle. Samples from both mouse lines were processed in parallel tubes.

[^{35}S]TBPS binding was studied in duplicate samples (about 200 μg protein per tube) during 90-min incubation at 22°C (room temperature) in 500 μl incubation volume in Tris-citrate buffer supplemented with 200 mM NaCl (35). [^{35}S]TBPS was used at a concentration of 6 nM. Unlabeled TBPS was added in saturation experiments to make up the concentration range, from 6–200 nM. Picrotoxinin at 10 μM was used to define the nonspecific binding. The effects of GABA antagonists bicuculline (50 μM) and SR 95531 (10 μM), a benzodiazepine agonist diazepam (10 μM), and ethanol (100 mM) were also studied. After incubation the bound ligand was separated from the free one by rapidly filtering the samples onto Whatman (GF/B) glass fibre filters using a Brandel M-48R filtration unit. The filters were rinsed twice with 5 ml ice-cold 10 mM Tris-HCl buffer (pH 7.4), air-dried, and immersed in 4 ml of Wallac Optiphase Hisafe II scintillation cocktail. The radioactivities were counted with a Wallac scintillation counter using external standardization.

[^{3}H]Ro 15-4513 binding at 10 nM was studied in the cerebellar membranes, as described in Taira et al. (53). The diazepam-insensitive binding was defined in the presence of 200 μM diazepam, and the nonspecific binding with 10 μM flumazenil. GABA stimulation of [^{3}H]flunitrazepam binding was carried out as described in Taira et al. A radioligand concentration of 1 nM was used, and the nonspecific binding was defined by 10 μM flumazenil. The GABA concentration ranged from 100 to 1 mM. The K_D and B_{max} values for the [^{35}S]TBPS binding and the EC_{50} values, maximal stimulations, and Hill coefficients for the GABA stimulation of [^{3}H]flunitrazepam binding were determined using nonlinear regression analysis with the GraphPad Inplot program.

RESULTS

Monoamines

The concentrations of NE, DA, and 5-HT, and the metabolites in the male and female frontal cortex, hypothalamus, and brain stem are shown in Figs. 1 and 2. The female mice exhibited higher concentrations of all these monoamines and metabolites in the frontal cortex and hypothalamus than the male mice (for statistical significances calculated using two-way ANOVA, see Fig. 1). In the brain stem, 5-HIAA levels were significantly higher in the females than the males. Furthermore, the ratio between DOPAC and DA in the hypothalamus and the ratio between 5-HIAA and 5-HT in the brain stem were higher in the females (for statistical significances, see Table 1). The ratios between DOPAC and DA, and between HVA and DA, were higher in the male than the female cortex (Table 1).

A comparison between transgenic TGFα and nontransgenic mice revealed no significant alterations in the levels of monoamines or metabolites in the males. The female TGFα mice exhibited significant elevations in the concentration of NE in the hypothalamus (Fisher's least-significant difference test: $p < 0.01$) and 5-HT in the cortex ($p < 0.001$) and brain stem ($p < 0.005$) compared with nontransgenic females (for statistical significances, see Fig. 1). The ratio between 5-HIAA and 5-HT, indicating the 5-HT turnover rate, was reduced in the male and female TGFα mice (Table 1). In the female transgenics, the reduction was significant in the frontal cortex ($p < 0.05$) and in the male transgenics, in the brain stem ($p < 0.05$).

Dopamine Transport

There were no gender differences in [^{3}H]GBR 12935 transporter binding in the frontal cortex or cerebellum in the nontransgenic CD-1 mice (Fig. 3). Among the transgenic mice, the binding of [^{3}H]GBR 12935 in the frontal cortex was 31% lower in the males than the females (Student's *t*-test: $t = 3.02$, $p < 0.04$). Both sexes of transgenic TGFα mice exhibited 26% lower values for [^{3}H]GBR 12935 transporter binding in the cerebella than the nontransgenic mice (Fig. 3).

5-HT Receptors

No differences in binding to 5-HT$_{1A}$ or 5-HT$_2$ receptors were found in the cortex or hippocampus between the transgenic and nontransgenic mice (Fig. 4). The ratio between 5-HT$_2$ and 5-HT$_{1A}$ receptors was slightly higher in the controls

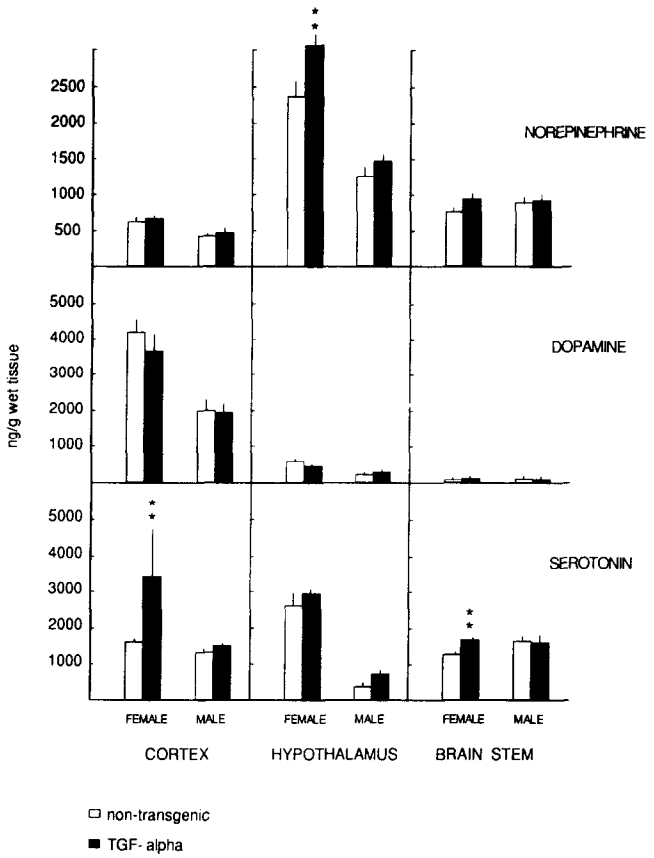

FIG. 1. Concentrations (nanogram per gram wet tissue) of monoamines in the brains of transgenic TGFα and nontransgenic control mice. Means + SEM of four to five animals per group are shown. Statistically significant differences are as follows. For the cortex: NE, between sexes, $F(1, 15) = 33.68$, $p < 0.0001$. DA, between sexes, $F(1, 14) = 35.14$, $p < 0.0001$. 5-HT, between sexes, $F(1, 15) = 16.63$, $p < 0.001$; between TGFα and nontransgenic mice, $F(1, 15) = 8.43$, $p < 0.01$; and between female transgenic and nontransgenic mice, $p < 0.001$. For the hypothalamus: NE, between sexes, $F(1, 15) = 68.18$, $p < 0.0001$; between TGFα and nontransgenic mice, $F(1, 15) = 13.12$, $p < 0.0025$; interaction between sex and genotype $F(1, 15) = 4.83$, $p < 0.04$; and between female transgenic and nontransgenic mice, $p < 0.01$. DA, between sexes, $F(1, 15) = 37.85$, $p < 0.0001$. 5-HT, between sexes, $F(1, 14) = 63.92$, $p < 0.0001$. For the brain stem: 5-HT, between female transgenic and nontransgenic mice, $p < 0.005$.

(mean ± SEM; 2.70 ± 0.14) than in the TGFα mice (2.45 ± 0.09) (Student's t-test: $t = 1.5$, $p < 0.16$).

GABA

The convulsant binding site on $GABA_A$ receptors, labeled by [^{35}S]TBPS, was reduced significantly less in the male TGFα mice after blockade of the $GABA_A$ agonist site by bicuculline (Student's t-test: $t = 2.57$, $p < 0.03$) and SR 95331 ($t = 2.12$, $p < 0.06$) than in the nontransgenic controls (Table 2). Although no significant differences were seen in the number or affinity of the convulsant [^{35}S]TBPS-labeled binding site between the two groups, the $B_{max/Kd}$ ratio was significantly higher in the male transgenic than in the control mice ($t = 2.66$, $p < 0.03$). GABA-stimulated [^{3}H]flunitrazepam binding to the forebrain hemispheres or [^{3}H]Ro 15-4513 binding to the cerebellar membranes did not differ between transgenic and nontransgenic mice (Table 2).

DISCUSSION

The association between TGFα and neurotransmitters is mostly unexplored. Alexi et al. (1) reported that TGFα stimulates DA uptake in cultured rat fetal dopaminergic neurons. In the present study, many alterations in the neurotransmission were found in the brains of transgenic mice that overexpress TGFα. The binding of the [^{3}H]GBR 12935-labeled DA transporter was lower in the frontal cortex and cerebellum in the transgenic TGFα mice than in their nontransgenic controls. The reduction in the frontal cortex was noted only in the male TGFα mice, which suggests a gender difference in DA transporter binding. The concentration of NE was also altered in a gender-specific manner. NE was elevated in the frontal cortex in the female TGFα, but not in the male TGFα mice, when compared with appropriate nontransgenic CD-1 controls. No alterations in the concentrations of DA or its metabolites, DOPAC and HVA, in the frontal cortex, hypothalamus, or brain stem were found.

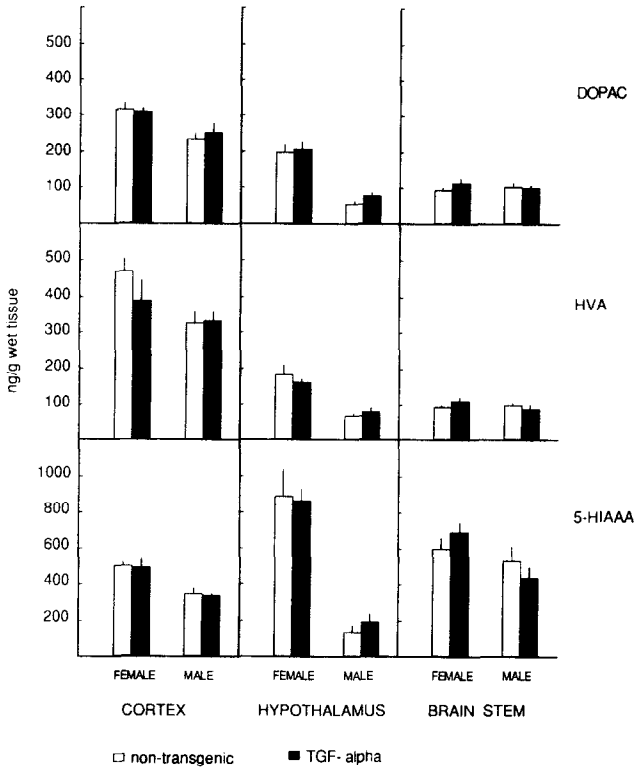

FIG. 2. Concentrations (nanogram per gram wet tissue) of monoamine metabolites in the brain of transgenic TGFα and nontransgenic control mice. Means + SEM of four to five animals per group are shown. Statistically significant differences are as follows. For the cortex: DOPAC, between sexes, $F(1, 15) = 13.91$, $p < 0.002$. HVA, between sexes, $F(1, 14) = 7.94$, $p < 0.01$. 5-HIAA, between sexes, $F(1, 16) = 6.27$, $p < 0.03$. For the hypothalamus: DOPAC, between sexes, $F(1, 15) = 69.35$, $p < 0.0001$. HVA,between sexes, $F(1, 15) = 35.32$, $p < 0.0001$. 5-HIAA, between sexes, $F(1, 14) = 65.15$, $p < 0.0001$. For the brain stem: 5-HIAA, between sexes, $F(1, 16) = 6.27$, $p < 0.03$.

TABLE 1

RATIOS BETWEEN MONOAMINES AND THEIR METABOLITES IN THE BRAIN OF TRANSGENIC TGFα AND NONTRANSGENIC CONTROL MICE

	Females		Males	
Brain Regions	Control	TGFα	Controls	TGFα
Cortex				
DOPAC/DA	0.07 ± 0.00	0.09 ± 0.01	0.12 ± 0.01	0.14 ± 0.01*
HVA/DA	0.12 ± 0.01	0.11 ± 0.01	0.17 ± 0.01	0.18 ± 0.02†
5-HIAA/5-HT	0.32 ± 0.03	0.20 ± 0.04	0.26 ± 0.02	0.23 ± 0.02‡
Hypothalamus				
DOPAC/DA	0.35 ± 0.02	0.44 ± 0.04	0.29 ± 0.02	0.32 ± 0.04§
HVA/DA	0.32 ± 0.05	0.34 ± 0.04	0.34 ± 0.02	0.33 ± 0.04
5-HIAA/5-HT	0.39 ± 0.02	0.34 ± 0.02	0.35 ± 0.05	0.29 ± 0.02‖
Brain stem				
DOPAC/DA	2.51 ± 0.46	2.37 ± 0.44	2.68 ± 0.38	2.81 ± 0.28
HVA/DA	2.34 ± 0.36	2.22 ± 0.42	2.63 ± 0.41	2.34 ± 0.42
5-HIAA/5-HT	0.48 ± 0.03	0.43 ± 0.04	0.32 ± 0.01	0.28 ± 0.02**

Means ± SEM of four to five animals per group are shown. Statistically significant differences:

*Between sexes, $F(1, 15) = 22.38, p < .0003$

†Between sexes, $F(1, 14) = 24.56, p < .0002$

‡Between TGFα and nontransgenic mice, $F(1, 16) = 9.34, p < .008$; between female transgenic and nontransgenic mice, $p < .05$

§Between sexes, $F(1, 15) = 8.15, p < .003$; between TGFα and nontransgenic mice, $F(1, 15) = 3.45, p < .08$

‖Between TGFα and nontransgenic mice, $F(1, 14) = 4.13, p < .06$

**Between sexes, $F(1, 16) = 28.58, p < .0001$; between male transgenic and nontransgenic mice, $p < .05$

Catecholamines are involved in the control of many behaviors. DA, for example, participates in the regulation of alcohol intake and the intake of other drugs of abuse (10,31). It has also been associated with depression, aggression, and locomotor activity (33,58,61). Thus, the increased alcohol intake, altered behavioral response to alcohol, high levels of aggression and locomotor activity, and lengthened immobility in the swim test in male TGFα mice (23,25) could have resulted from reduced DA transport in the frontal cortex.

Similarly to catecholamines, the 5-HT system was altered in a gender-specific manner in the transgenic TGFα mice. The concentration of 5-HT was significantly elevated in the frontal cortex and brain stem of the female TGFα mice. No such increase was seen in the male transgenics. In the male TGFα mice, the 5-HIAA/5-HT ratio was low in the brain stem, which suggests a reduced 5-HT turnover. This ratio was also reduced in the cortex of the female TGFα mice. However, in the males, the 5-HIAA/5-HT ratio was reduced because of a low brain-stem 5-HIAA concentration. In the females, the reduction was caused by increased levels of 5-HT; 5-HIAA levels were normal. Therefore, it may be that in female TGFα mice the synthesis of 5-HT is increased or uptake inhibited, rather than that their 5-HT turnover is reduced.

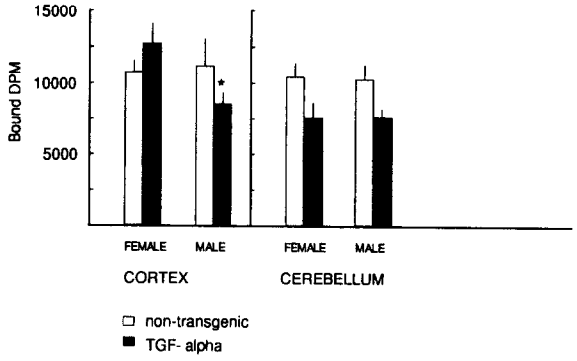

FIG. 3. [^{3}H]GBR 12935 binding to dopamine transporter in the frontal cortex and cerebellum of female and male transgenic TGFα and nontransgenic mice. Each group represents the mean ± SD of five individually assayed mouse brains. Statistical significance was evaluated by a two-tailed t-test. *$p < 0.04$ between male TGFα mice.

Serotonin is considered to be critical in affective disorders. Among the behaviors that are affected by manipulations of serotonergic functions are voluntary alcohol intake (51), depression (17,21,62), and aggression (45,59,60). Specifically, low levels of 5-HT and 5-HIAA are typically found in depressive (20,40,49,62) and aggressive individuals (20,36,40,48,60) and alcohol abusers (50,55). We have found that the 5-HT precursor, tryptophan; 5-HT uptake inhibitors (28); and 5-HT$_{1A}$ receptor agonist buspirone and 5-HT$_2$ receptor antagonist ketanserin (unpublished data) shorten immobility in the swim test in the male TGFα. 5-HT uptake inhibitors (28) and receptor agonists and antagonists (unpublished data) are also effective in reducing aggressive behavior in these mice. Thus, the behavioral (25,28) and neurochemical findings in the male TGFα mice support the hypothesis that impaired 5-HT function may be associated with increased aggression, depressive behavior, and alcohol intake.

In females, the reduced time spent in exhibiting aggressive behavior and immobility in the swim test (24) might be due to elevated levels of 5-HT in the brain. Clinical data have shown that the depletion of brain 5-HT concentrations with a low tryptophan diet induces depressive behavior in humans (65). L-tryptophan is effective in the treatment of mild to moderate

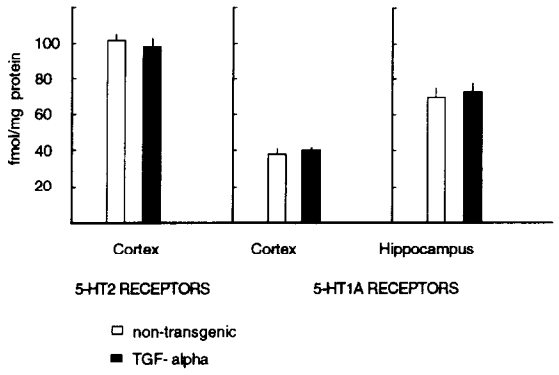

FIG. 4. 5-HT_2 and 5-HT_{1A} receptors in the cortex of male transgenic TGFα mice and nontransgenic CD-1 controls. Values are means ± SEM of seven to nine animals per group.

depression (26,64). Our studies indicate that tryptophan reverses lengthened immobility in the swim-test model of depressive behavior in mice preexposed to various stressors (28).

$GABA_A$ receptors are heterogenous in their properties. These receptors exhibit differential expression of the α subunit variants that cause the receptors to have different benzodiazepine ligand-binding specificities (41) and different sensitivities to their natural transmitter GABA (34,35). The present ligand-binding comparisons between the transgenic and nontransgenic mice did not suggest any drastic alterations in general $GABA_A$ receptor populations, or in the allosteric coupling between GABA and benzodiazepine sites within the receptor complex. The significant differences between the transgenics and nontransgenics in the effects of $GABA_A$ antagonists on convulsant binding and in the binding site density/affinity ratios could be due to differences in the endogenous agonist (GABA) concentration and/or in receptor subtype populations. Both phenomena might lead to an altered GABAergic neurotransmission. Because many behavioral disorders including anxiety (15), increased locomotor activity (18), and increased voluntary alcohol intake (4) are associated with alterations in GABAergic transmission, the behavioral characteristics of the male TGFα may result from changes in this neurotransmitter system.

TABLE 2

RATIOS BINDING INDICATORS OF $GABA_A$ RECEPTOR FUNCTION IN THE MALE TRANSGENIC TGFα MICE AND NONTRANSGENIC CD-1 CONTROLS

	Controls	TGFα
[^{3}H]TBPS binding for forebrain hemispheres		
K_D, nM	73.5 ± 5.0	78.6 ± 6
B_{max}, pmol/mg protein	7.60 ± 0.21	6.92 ± 0.33
Bicuculline 50 μM, % of basal	79 ± 2	90 ± 3*
SR 95331 10 μM, % of basal	92 ± 2	101 ± 3†
Ethanol 100 mM, % of basal	76 ± 1	76 ± 1
Diazepam 10 μM, % of basal	58 ± 2	54 ± 2
[^{3}H]Flunitrazepam binding to forebrain hemispheres		
Basal binding, pmol/mg protein	2.27 ± 0.19	2.21 ± 0.14
EC_{50} for GABA stimulation, μM	2.18 ± 0.45	2.82 ± 0.71
Maximal GABA stim., % over basal	58 ± 6	69 ± 2
Hill coefficient	0.99 ± 0.08	0.79 ± 0.07
[^{3}H]Ro 15-4513 binding to cerebellar membranes		
Total binding, pmol/mg protein	3.05 ± 0.17	2.88 ± 0.06
Diazepam-sensitive binding	2.34 ± 0.13	2.17 ± 0.04
Diazepam-insensitive binding	0.68 ± 0.04	0.71 ± 0.02

Values are means ± SEM of six animals per group. Statistically significant differences (*t*-test):
*$p < 0.03$.
†$p < 0.06$.

In addition to differences between transgenic TGFα and nontransgenic CD-1 mice, we found many gender-specific differences in the brain concentrations of monoamines and metabolites. The monoamine levels were higher in the female than in the male brain. These findings are consistent with previous data showing sex differences in the brain catecholamine and serotonin contents (5,7,19). In particular, the levels of 5-HT and 5-HIAA are reported to be higher in the female than male hypothalamus (38), and the catalytic activity of enzymes converting catecholamines from precursor tyrosine is higher in the female brain (9). It is interesting that the concentrations of monoamines were slightly, but not significantly, higher in the male TGFα mouse brain than in the nontransgenic male one, suggesting feminization of the male transgenic brain.

One explanation for the alterations in certain neurotransmitters in the brains of transgenic TGFα mice could involve the role of this growth factor in development. For example, TGFα accelerates tooth eruption and eyelid opening in newborn mice (52,54), possibly through mechanisms related to the estrogen that is elevated in plasma of TGFα mice (24,25). In female rats, TGFα induces release of gonadotrophin releasing hormone (GnRH) from the hypothalamus (46), but importantly, TGFα can activate estrogenic pathways even in the absence of E_2 (11). Estrogens exert growth-promoting effects on the differentiation of target neurons and their neurites in several areas in the brain (56). It has also been suggested that estrogen and nerve growth factor act on the same neuron to regulate the expression of specific genes that may influence many neuronal functions (57). At the neurotransmitter level, steroids have been shown to regulate expression of genes coding for neurotransmitter receptors, neuropeptides, G proteins, and enzymes involved in neurochemical metabolism (2). Physiologic concentrations of endogenous steroids are potent modulators of the GABA/benzodiazepine receptor-chloride ionophore (42). Furthermore, steroids modify the expression of 5-HT_{1A}-coupled Gi-proteins, resulting in altered sensitivity of 5-HT_{1A}-mediated signal transduction (39).

In summary, the behavioral alterations seen in transgenic TGFα mice could result from the overexpression of this growth factor, increased plasma E_2 levels, and/or altered neurotransmission. The present data suggest that overexpression of TGFα is associated with elevated concentrations of NE and 5-HT in female transgenic mice. In male transgenic mice, TGFα appears to be linked to reduced 5-HT turnover, reduced DA transmission, and altered endogenous GABA concentration or $GABA_A$-receptor populations. It remains to be determined whether the gender-specific alterations in neurotransmission in TGFα mice are caused by the effects of this growth factor on gonadal hormones, and whether the neurotransmitter alterations are responsible for the altered sexually dimorphic nonreproductive behaviors between male and female transgenic mice (23).

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