

Testosterone and free thyroxin blood in congenitally acallosal male BALB/cCF mice

Carlos Alberto Mourão Júnior^{1,2}, Sergio Luís Schmidt³ & Alex Christian Manhães³

¹ Departamento de Fisiologia, Instituto de Ciências Biológicas, Universidade Federal de Juiz de Fora, BRAZIL.

² Programa de Pós-Graduação em Endocrinologia, Universidade Federal de São Paulo, BRAZIL.

³ Laboratório de Neurofisiologia, Departamento de Ciências Fisiológicas, Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro, BRAZIL.

Correspondence to: Laboratório de Neurofisiologia,
Departamento de Ciências Fisiológicas,
IBRAG, Universidade do Estado do Rio de Janeiro,
Boulevard 28 de Setembro, 87, fundos, 5 andar,
Vila Isabel, Rio de Janeiro, RJ, BRAZIL
TEL: +55 21 2055-1030 / 2587-6498
FAX: +55 21 2552-2247
EMAIL: amanhaes@uerj.br

Submitted: July 7, 2003

Accepted: November 17, 2003

Key words: testosterone; thyroxin; corpus callosum; mice

Neuroendocrinol Lett 2003; 24(6):459-462 NEL240603A13 Copyright © Neuroendocrinology Letters www.nel.edu

Abstract

OBJECTIVES: Approximately 20% of BALB/cCF mice are born with partial or total absence of the corpus callosum. Here, we analyzed testosterone and free thyroxin blood levels in adult male mice of this strain in order to see if these hormones are related to the incidence of callosal defects.

METHODS: Blood collected from the axillary blood vessels of 12 normal and 10 acallosal deeply anesthetized adult male mice was used in order to determine testosterone and free thyroxin levels through chemiluminescence (IMMULITE, Diagnostics Products Corporation, USA).

RESULTS: No significant difference (one-way ANOVA: $F = 0.11$, $df = 1$, $p > 0.10$) was found between normal ($\bar{X} = 1.95$, $SD = 0.62$) and acallosal ($\bar{X} = 1.86$, $SD = 0.62$) mice for free thyroxin level. On the other hand, in those mice that had detectable testosterone levels (above 0.2 ng/ml), a significant difference was found ($t = 2.8$, $df = 6.06$, $p = 0.03$): normal mice ($n = 7$, $\bar{X} = 8.73$, $SD = 7.64$) had a higher level than acallosal mice ($n = 4$, $\bar{X} = 0.62$, $SD = 0.41$).

CONCLUSIONS: The present results indicate that the incidence of callosal agenesis is not related to free thyroxin levels in the blood of adult BALB/cCF mice. On the other hand, in spite of the fact that low testosterone levels seems to be frequent in male mice of this strain, acallosal mice tend to have lower levels of this hormone than normal mice.

Introduction

In adult animals, the corpus callosum (CC) is responsible for the functional integration of the hemispheres by allowing a considerable amount of information to be exchanged between them [1]. In developing animals, the CC is additionally involved in the establishment of neocortical morphology [2] and brain asymmetries [3,4]. Considering the importance of this commissure for brain development, a number of reports have addressed the events directly related to the ontogenetic development of the CC [1]. One area that has received a considerable attention is that of the effects of hormones on callosal development and maturation. For instance, it has been recently demonstrated that the incidence of callosal agenesis in female mice of the BALB/cCF strain is higher than in males [5]. Additionally, experimental manipulation of hormonal levels during gestational and postnatal development in rats indicated that testosterone and ovarian hormones have a significant effect on adult callosal morphology [6, 7, 8]. In humans, it is also known that the incidence of callosal agenesis varies according to sex [9] and that the regional morphology of the human CC is affected by testosterone [10]. As for thyroxin, it has been demonstrated that hypothyroid rats present altered cortical citoarchitecture and problems in neuronal maturation and development [11,12]. Specifically regarding the CC, it has been shown that hypothyroid rats present an impairment on myelin compaction [13] accompanied by a significant reduction in the number of myelinated axons [14] and a disruption of the normal pattern of callosal connections in cortical areas [15].

In the present study, we tested the hypothesis that the incidence of callosal agenesis in BALB/cCF mice, which present approximately 20% of animals with partial or total callosal agenesis, may be associated with the blood levels of testosterone and thyroxin.

Material and methods

Subjects were twenty-two adult male BALB/cCF mice. All mice were bred and maintained in our laboratory. The animals were kept in a temperature-controlled room on a 12 h light/dark cycle. All mice were separated from their progenitors in the 30th postnatal day and males were separated from females. Littermates were housed together up to the day in which the experiment was carried out (between the 60th and 75th postnatal days). Access to food and water was ad libitum. This study was carried out under institutional approval, avoiding animal suffering.

After being deeply anesthetized with ether, thoracic dissection was performed so that blood could be drawn from the axillary blood vessels with the aid of a Pasteur's pipette. Blood samples were then transferred to dry disposable test tubes, each one receiving an identification code. Immediately after sampling, all mice were transcardially perfused with saline (0.9 % NaCl), followed by 4% phosphate-buffered paraformaldehyde solution (pH 7.4). The brains were then removed from the skulls with the aid of a dissection microscope (magnification: 10X) and the hemispheres were separated along the midsagittal plane. Qualitative analyses of the morphology of the CC were performed so that only the blood samples from animals with clearly normal (12 mice) or abnormal CC (10 mice) were selected for hormone level measurements. Selected brains were stained with golden chloride [16]. As a result of the staining procedure, the inter-hemispheric commissures (including the CC) became dark brown in color, which contrasted with the light background. Photographs of the midsagittal planes of the stained hemispheres were taken from all animals (Figure 1).

Blood samples were kept chilled in heat-insulated containers until the measurements of free thyroxin and testosterone were performed (within 24h of blood sampling). The measurements were performed using a fully automated method of chemiluminescence (IMMULITE, Diagnostics Products Corporation, USA), which rules out inter and intra-assay variations. It must be pointed out that this method is not susceptible to interference from hemolysis. Some animals, in both groups, presented testosterone levels that were lower than the minimum level that the equipment was capable of measuring (0.2 ng/ml; in these cases, the measurements were repeated and confirmed). In order to allow comparisons between groups using all animals, we performed a data transformation [17] in which animals with undetectable levels up to 0.99 ng/ml received

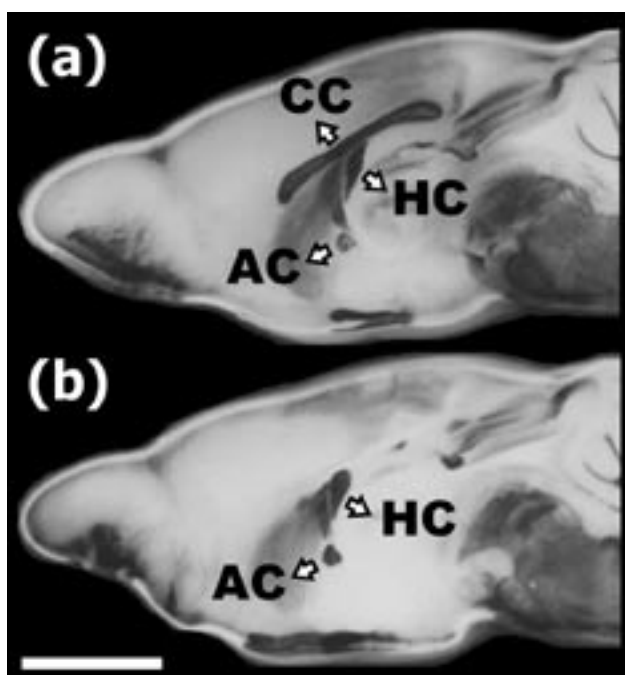


Figure 1 – Midsagittal golden chloride-stained hemispheres from a normal (A) and an acallosal (B) mice. Note in A, the presence of the corpus callosum (arrowheads) and in B, the absence of this commissure. CC = corpus callosum, HC = hippocampal commissure, AC = anterior commissure. Calibration A = B = 3 mm.

a score 0. Animals with levels between 1.00 up to 1.99 ng/ml received a score 1. The same transformation procedure was applied in subsequent intervals.

Results

Table I presents testosterone and free thyroxin (free T4) plasma levels for all mice that were used in the present study.

The difference between the distributions of testosterone level scores of normal and acallosal animals approached significance (Mann-Whitney test: $Z = 1.76$, $p = 0.07$). Since the percentage of animals presenting detectable levels of testosterone did not differ between groups (normal: 58%, acallosal: 40%; $\chi^2 = 0.73$, $df = 1$, $p > 0.10$), an analysis of testosterone levels in those animals which had a detectable quantity of the hormone was carried out and indicated that a significant difference (t-student: $t = 2.8$, $df = 6.06$, $p = 0.03$) was present: normal animals ($\bar{X} = 8.73$, $SD = 7.64$) had a higher mean level of testosterone than acallosal ones ($\bar{X} = 0.62$, $SD = 0.41$).

No significant difference was found between normal ($\bar{X} = 1.95$, $SD = 0.62$) and acallosal ($\bar{X} = 1.87$, $SD = 0.62$) mice regarding free T4 measurements (one-way ANOVA: $F = 0.11$, $df = 1$, $p > 0.10$).

No significant correlation was found between testosterone level scores and free thyroxin levels ($n = 22$; Kendall's tau = 0.06, $p > 0.10$).

Discussion

Sex differences in the incidence of callosal agenesis in BALB/cCF mice, females having twice as much abnormalities in CC morphology than males, suggested that sexual hormones played an important role in the early stages of callosal development [5]. Two possibilities exist that might explain the effects of sexual hormones: firstly, the lack of specific hormones could directly affect the genesis and/or short term viability of developing callosal neurons, resulting in a severely reduced number of axons crossing the midline. However, this possibility is rendered unlikely in the BALB/cCF strain due to the existence of an aberrant longitudinal fiber bundle that can be seen running rostro-caudally underneath the cortical white matter of adult acallosal mice [18]. This fiber bundle, known as Probst bundle, is comprised of axons that would constitute the CC but were unable to cross the midline, establishing connections in the ipsilateral neocortex. As for the second possibility, hormones could affect one or several of the developmental stages that direct callosal axons to the midline and, subsequently, allow callosal fibers to enter the contralateral hemisphere. Callosal axons are guided along their paths by midline cell populations such as those present in the glial wedge, indusium griseum glia and glial sling [1, 19]. The glial sling, for example, provides the structural support upon which callosal axons actually cross the midline and its cells can be seen around the 17th gestational day (E17) [1]. It has already been demonstrated that the glial sling

Table I.

Testosterone and free T4 blood levels in BALB/cCF mice

| Mice | Corpus Callosum | Testosterone | free T4 |
|------|-----------------|----------------------|---------|
| 1 | Abnormal | < 0.2 (undetectable) | 1.0 |
| 2 | Abnormal | < 0.2 (undetectable) | 2.0 |
| 3 | Abnormal | < 0.2 (undetectable) | 0.8 |
| 4 | Abnormal | < 0.2 (undetectable) | 1.7 |
| 5 | Abnormal | < 0.2 (undetectable) | 1.9 |
| 6 | Abnormal | < 0.2 (undetectable) | 2.4 |
| 7 | Abnormal | 0.24 | 1.8 |
| 8 | Abnormal | 0.46 | 2.8 |
| 9 | Abnormal | 0.58 | 2.5 |
| 10 | Abnormal | 1.20 | 1.7 |
| 11 | Normal | < 0.2 (undetectable) | 1.3 |
| 12 | Normal | < 0.2 (undetectable) | 1.2 |
| 13 | Normal | < 0.2 (undetectable) | 1.1 |
| 14 | Normal | < 0.2 (undetectable) | 1.9 |
| 15 | Normal | < 0.2 (undetectable) | 2.6 |
| 16 | Normal | 0.27 | 2.6 |
| 17 | Normal | 0.59 | 2.6 |
| 18 | Normal | 2.83 | 1.1 |
| 19 | Normal | 8.78 | 2.2 |
| 20 | Normal | 14.70 | 1.9 |
| 21 | Normal | 15.20 | 2.5 |
| 22 | Normal | 18.77 | 2.4 |

does not form in mice that present CC defects. Taking into account that testosterone production can be observed around E14-E15 and that the highest testosterone secretory capacity can be reached as early as E17 in mice [20], it is possible to speculate that testosterone levels in the blood interfere with this midline cell population in such a way that it renders the crossing difficult or impossible, leading to an abnormal or absent CC. In fact, despite the observed differences in testosterone levels between acallosal and normal mice in the present study, our results indicated that a considerable number of mice that have a normal CC also have a very low (even undetectable) level of this hormone in the blood. It must be pointed out, however, that most BALB/cCF mice do present a significant delay in the formation of the CC [21]. However, a normally sized CC can be observed in the adult mice [21] due to the employment of strategies such as the use of the dorsal hippocampal commissure as a support for the crossing.

Regarding thyroxin, previous reports have demonstrated that this hormone is relevant to several aspects of the development and maturation of neurons [11, 12]. Of particular interest to the present study are the reports that address the effects of hypothyroidism in the number of CC axons. It has been demonstrated that rats rendered hypothyroid at E14 present a se-

verely reduced number of myelinated CC axons at adulthood compared to normal rats [13]. This reduction is accompanied by indications that callosal axons are arrested in an immature stage of development [12, 13, 14]. Our results seem to indicate that there is no association between callosal agenesis and thyroxin blood levels in BALB/cCF mice, suggesting that it is not the lack of maturation of the callosal axons that is responsible for the altered adult CC morphology.

In conclusion, our results indicate that callosal agenesis in adult BALB/cCF mice is related to low blood levels of testosterone and that there is no direct association between callosal agenesis and blood levels of thyroxin.

REFERENCES

- 1 Lent R, Schmidt SL. The ontogenesis of the forebrain commissures and the determination of brain asymmetries. *Prog Neurobiol* 1993; **40**:249–76.
- 2 Abreu-Villaça Y, Silva, WC, Manhães AC, Schmidt, SL. The effect of corpus callosum agenesis on neocortical thickness and neuronal density of BALB/cCF mice. *Brain Res Bull* 2002; **58**:411–16.
- 3 Schmidt SL, Caparelli-Dâquer EM. The effects of total and partial callosal agenesis on the development of morphological brain asymmetries in the BALB/cCF mouse. *Exp Neurol* 1989; **104**:172–180.
- 4 Schmidt SL, Manhães AC, de Moraes VZ. The effects of total and partial callosal agenesis on the development of paw preference performance in the BALB/cCF mouse. *Brain Res* 1991; **545**:123–130.
- 5 Manhaes AC, Medina AE, Schmidt SL. Sex differences in the incidence of total callosal agenesis in BALB/cCF mice. *Neurosci Lett* 2002; **325**:159–62.
- 6 Bimonte HA, Fitch RH, Denenberg VH. Adult ovary transfer counteracts the callosal enlargement resulting from prepubertal ovariectomy. *Brain Res* 2000; **872**:254–7.
- 7 Bimonte HA, Mack CM, Stavnezer AJ, Denenberg VH. Ovarian hormones can organize the rat corpus callosum in adulthood. *Dev Brain Res* 2000; **121**:169–77.
- 8 Mack CM, McGivern, RF, Hyde, LA, Denenberg, VH. Absence of postnatal testosterone fails to demasculinize the male rat's corpus callosum. *Dev Brain Res* 1996; **95**:252–5.
- 9 Goodyear PW, Bannister CM, Russell S, Rimmer S. Outcome in prenatally diagnosed fetal agenesis of the corpus callosum. *Fetal Diagn Ther* 2001; **16**:139–145.
- 10 Moffat SD, Hampson E, Wickett JC, Vernon PA, Lee, DH. Testosterone is correlated with regional morphology of the human corpus callosum. *Brain Res* 1997; **767**:297–304.
- 11 Berbel P, Escobar del Rey F, Morreale de Escobar G, Ruiz-Marcos A. Effect of hypothyroidism on the development of cortical dendritic spines. An electron microscopic study. *Ann Endocrinol* 1983; **44**:16A.
- 12 Gravel C, Sasseville R, Hawkes R. Maturation of the corpus callosum of the rat: II. Influence of thyroid hormones on the number and maturation of axons. *J Comp Neurol* 1990; **291**:147–161.
- 13 Barradas PC, Ferraz AS, Ferreira AA, Daumas RP, Moura EG. 2'3' cyclic nucleotide 3'phosphodiesterase immunochemistry shows an impairment on myelin compaction in hypothyroid rats. *Int J Devl Neuroscience* 2000; **18**:887–892.
- 14 Berbel P, Guadaño-Ferraz A, Angulo A, Cerezo JR. Role of thyroid hormones in the maturation of interhemispheric connections in rats. *Behav Brain Res* 1994; **64**:9–14.
- 15 Gravel C, Hawkes R. Maturation of the corpus callosum of the rat: I. Influence of thyroid hormones on the topography of callosal projections. *J Comp Neurol* 1990; **291**:128–146.
- 16 Schmued LC. A rapid sensitive histochemical stain for myelin in frozen brain sections. *J Histochem Cytochem* 1990; **38**:717–720.
- 17 Sokal RR, Rohlf, FJ. *Biometry*. San Francisco (CA): Freeman; 1981.
- 18 Ozaki HS, Shimada M. The fibers which course within the Probst's longitudinal bundle seen in the brain of a congenitally acallosal mouse: a study with the horseradish peroxidase technique. *Brain Res* 1988; **441**:5–14.
- 19 Shu T, Richards LJ. Cortical axon guidance by the glial wedge during the development of the corpus callosum. *J Neurosci* 2001; **21**:2749–58.
- 20 de Ruiter AJH, Feitsma LE, Keijsers JN, Koolhaas JM, van Oortmerssen GA, Bohus B. Differential perinatal testosterone capacity of wild house mice testes is related to aggressiveness in adulthood. *Horm Behav* 1993; **27**:231–9.
- 21 Wahlsten D. Defects of the fetal forebrain in mice with hereditary agenesis of the corpus callosum. *J Comp Neurol* 1987; **262**:227–241.