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Behavioural Brain Research 107 (2000) 35–43

BEHAVIOURAL BRAIN RESEARCH

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Research report

Early androgen treatment decreases cognitive function and catecholamine innervation in an animal model of ADHD

Jean A. King *, Russell A. Barkley, Yvon Delville, Craig F. Ferris

Department Of Psychiatry, University Of Massachusetts Medical School, 55 Lake Ave. North, Worcester, MA 01655, USA

Received 14 January 1999; received in revised form 25 June 1999; accepted 28 June 1999

Abstract

The spontaneously hypertensive rat (SHR) has been used as an animal model of attention deficit hyperactivity disorder (ADHD). The present study was designed to determine whether exposure to elevated androgen levels early in development demonstrated impairments in cognitive functioning, neuroendocrine control, and brain development parallel to those seen in ADHD children. The animals (SHR and Wistar (WKY) controls) were implanted with testosterone on postnatal day 10 and tested for behavior in a spatial cognition paradigm on postnatal day 45. Plasma samples were collected for determination of adrenocorticotrophin hormone (ACTH) and corticosterone levels as indicators of the basal tone of the pituitary–adrenal neuroendocrine axis. In addition, the density of tyrosine hydroxylase-immunoreactive fibers (an indicator of catecholamine innervation) in the frontal cortex was compared between animals. The current data show that early testosterone treatment in SHR animals resulted in additional deficits in spatial memory in the water maze, but was ineffective in altering the response of WKY animals. Furthermore, SHR rats had high basal ACTH and low corticosterone levels that may indicate a dysfunctional stress axis similar to other reports in humans with persistent ADHD. Finally, there was a further suppression of tyrosine hydroxylase-immunoreactivity in the frontal cortex of androgen-treated SHR rats. These results support the hypothesis that early androgen treatment may support the neurobiology of animals with genetic predisposition to hyperactivity, impulsivity and inattention in a manner consistent with the enhanced expression of ADHD-like behaviors.

Keywords: ADHD; Catecholamines; Cognition; Development; H–P–A axis; Testosterone

1. Background

Attention deficit hyperactivity disorder (ADD/+H or ADHD) affects approximately 2–5% of grade-school children [3,4,37]. The symptoms include hyperactivity, impulsivity, and the inability to sustain attention. The etiology of ADHD remains unclear, but heredity apparently plays a major role [3]. Boys appear more likely to be affected by ADHD; the sex ratio ranges from 3:1 to 8:1, boys to girls [19,38]. The two most obvious possibilities for the development of a preponderance of ADHD in males are genetic patterns of inheritance and hormonal conditions such as higher testosterone levels. During the prenatal and early postnatal period there are significantly higher levels of testosterone in males in a number of species, including man [6,8,10,11,20,24,27,35]. These elevated testosterone levels are responsible for gender-specific organizational effects on the developing nervous system [2,13,23] through modulation of neurotransmitters like dopamine [14,29,36], which has been suggested to play a pivotal role in the neurobiology of ADHD [26]. In particular, the dopamine system in the frontal cortex has been implicated in the neurobiology of ADHD [3,5,25,26]. Testosterone has been shown to affect dopamine activity in the mesolimbic system [14], to promote neurite outgrowth in the mesencephalic neurons [29], and to influence development of dopamine neurons in the frontal cortex [38]. But the role of testosterone in the development of cognitive functioning,

* Corresponding author. Tel.: +1-508-856-4979; fax: +1-508-856-6426.

E-mail address: jking@bangate.ummed.edu (J.A. King)

0166-4328/00/$ - see front matter
PII: S0166-4328(99)00113-8

neuroendocrine parameters, and dopamine innervation in the frontal cortex of an animal model of ADHD remains unknown. Several animal model of ADHD have been proposed, including rats with neurotoxic brain lesions [1], dopamine alteration [33] and spontaneous hypertension [9,22,30,32] and mice with gene deletion [12]. Although each model has its own unique limitations, the SHR (spontaneously hypertensive rat) model has been proposed as an acceptable and most frequently used animal model of ADHD [7,9,22,32], we tested the hypothesis that testosterone may differentially affect individuals predisposed to ADHD versus those not predisposed. Consequently, we assessed cognitive performance, neuroendocrine parameters, and changes in tyrosine hydroxylase-containing fibers (an indicator of dopamine and norepinephrine neurons) in SHR animals which had received early testosterone treatment. The results suggest that excess testosterone early in development may predispose genetically vulnerable male animals to ADHD-like behaviors and may explain the gender differences observed in humans.

2. Methods

2.1. Animals

Pregnant rats (SHR and Wistar-Kyoto, WKY, $n = 4 + 4$) were obtained from Charles River Breeding Laboratories (Wilmington, MA) during their first week of gestation. The animals were housed singly under stimulating photoperiod (12L, 12D; lights on at 20:00) and received food and water ad libitum. All litters were culled to eight after delivery. Only male rats were used in the study. Pups were housed with dams and weaned on gestation day 21. All animals were then group-housed (four animals per cage) and tested on postnatal day 45.

2.2. Hormone treatment

With the working hypothesis that high levels of androgens in the frontal cortex maybe important to ADHD expression, treatment with testosterone was intracranially administered to bypass the negative feedback on the hypothalamic–pituitary–gonadal axis. On postnatal day 10, all animals were stereotaxically implanted with testosterone (10 ng; SHR, $n = 6$; WKY, $n = 6$) or cholesterol pellets (10 ng; SHR, $n = 6$; WKY, $n = 6$). The surgery was performed under ketamine (50 mg/kg; Sigma, St. Louis, MO) anesthesia. The stereotaxic coordinates were: 4.8 mm anterior to bregma, 2.3 mm lateral to the midsagittal suture, and 2.2 mm below dura. The incisor bar was held at 0 mm with the interaural line.

2.3. Behavior

SHR animals were tested in a cue-based spatial cognition paradigm that assessed the rat's ability to use visual cues outside the pool to find an escape platform located beneath the surface of the water. Beginning on postnatal day 45, animals were removed from their home cage at 11:00 h each day for assessment of behavior in the Morris water maze. Training and testing in the Morris water maze consisted of a habituation phase (performed on the first day), an acquisition phase with testing trials (performed between the second and fifth days) and a final testing phase (performed on the sixth day). The maze was set up with a swimming pool diameter of 1.52 m. The pool was divided into four quadrants (NE, SE, SW, NW) and visual cues were placed at these quadrants both on the side of the pool and on the curtain surrounding the water maze. A video camera (Computar, Model FC62B) was affixed to the ceiling exactly above the center of the pool. The camera was connected to a monitor for viewing ongoing experiments, a VCR for recording the sessions, and a computer with tracker (HVS Image VP118) for scoring of latencies and distances traveled. The software package used for scoring data was HVS Water for Windows, version 797 (HVS Image, Hampton, UK). During the habituation phase (day 1), each rat was placed in the water maze and allowed to swim without the platform in place for 90 s. Rats were placed in a holding tank for drying out (60 s) and then returned to their home cages. Then, training (acquisition period/testing trials, days 2, 3, 4,5) was performed with the platform submerged 2 cm below water level. Each of the three trials began with placing the rat into the pool facing the walls at either the north, south, east or west position. The starting quadrant in the pool was different for each rat. On each of the three trials if the rats did not find the platform within 120 s, the experimenter guided them to it. The rat remained on platform for 45 s to allow orientation to visual cues. On the final day of testing (day 6), the platform was submerged (2 cm) and the starting position was alternated for each of five trials. Latencies to reach the platform (s) was recorded for each rat on days 2–6.

2.4. Hormone radioimmunoassays

Researchers have shown differences in basal neuroendocrine functioning in ADHD [16] which maybe similar to those found in the SHR animal model. To test this premise hormonal radioimmunoassays were performed. Twenty-four hours after the behavioral tests, animals ($n = 6$ per group) were quickly sacrificed (by decapitation) and trunk blood was collected. The blood samples were placed on ice, centrifuged, and plasma removed. The plasma was stored at -80°C until the assays were

performed. ACTH was measured in plasma samples with a kit obtained from ICN Pharmaceutical (Costa Mesa, CA). This antiserum to ACTH crossreacts less than 0.1% with β-endorphin. Corticosterone was measured in plasma samples with a kit obtained from ICN Pharmaceutical. The antiserum to corticosterone crossreacts less than 0.01% with testosterone and androstenedione.

2.5. Immunocytochemistry

Since other researchers have implicated changes in dopamine in the frontal cortex in the neurobiology of ADHD [3,26], immunocytochemical methods were used to assess changes in tyrosine hydroxylase immunoreactivity (a marker for dopamine and norepinephrine fibers). The animals ($n = 4$ per group) were perfused transcardially, first with 0.9% saline containing 1% sodium nitrite to dilate blood vessels, followed by 4% paraformaldehyde and 2.5% acrolein in 0.1 M potassium phosphate-buffered saline (KPBS), and again with saline to eliminate excess fixatives. The brains were then removed, immersed in 20% sucrose in KPBS, and kept at 4°C overnight. The perfusions were performed under sodium pentobarbital anesthesia (Nembutal, 35 mg/kg, Abbott Laboratories, North Chicago, IL), after an intracardiac injection of heparin (5000 U in 1 ml saline). Later, the brains were sectioned coronally at 50 μm on a freezing microtome, and the sections saved in cryoprotectant [40] at −20°C until used for immunocytochemistry.

Immunocytochemistry was performed as follows. The sections were washed in 0.05M Tris-buffered saline (TBS, pH 7.6) before incubation in 1% sodium borohydrite to remove residual aldehydes. The sections were washed again and incubated for 30 min in a solution containing 20% goat serum, 1% hydrogen peroxide, and 0.3% Triton X-100 (respectively, to reduce non-specific labeling, eliminate endogenous peroxidase, and permeabilize the tissue). The sections were then incubated for 1 h at 37°C in the primary antibody (mouse monoclonal anti-tyrosine hydroxylase, 1/10 000, Sigma, St Louis, MO) in the presence of 2% goat serum and 0.3% Triton X-100. After washes, the sections were incubated for 45 min in the secondary antibody (biotinylated goat anti-mouse IgG, 7.5 μg/ml, Vector Laboratories, Burlingame, CA). Then, the sections were washed again and placed in the tertiary incubation (Vectastain ABC Elite kit, Vector Labs) for another 45 min before being washed and labeled with nickel-conjugated diaminobenzidine. This led to a blue/black precipitate labeling of all known dopaminergic cell groups and projections. All solutions were prepared with 0.05 M TBS. This procedure is optimal for the labeling of fibers and neurons, and results in a near absence of background labeling. Omission of the primary antibody prevented all immunostaining.

2.6. Fiber density

Tyrosine hydroxylase-immunoreactive (TH-ir) fibers were observed in distinct areas within the brain. The distributions of TH-ir fibers were compared between the groups of animals. The density of TH-ir fibers within selected areas was estimated in digitized images using the IMAGE software (Version 1.56, NIH, Bethesda, MD). The light and camera settings were kept at a same level of darkness for all slides and selected areas to minimize background variations. The areas selected included the frontal cortex and the nucleus accumbens. Fiber density was analyzed by gray-level thresholding [34] in digitized images using the IMAGE software (Version 1.56, NIH, Bethesda, MD) and obtained from a video camera (TM-745, Pulnix America, distributed by Motion Analysis, Eugene, OR) mounted on a microscope. The images were imported on a MacIntosh computer with a frame grabber (LG-3, Scion, Walkersville, MD). A standard sample (square of 0.04 mm^2) was placed within the selected areas. The selected areas were similar for all animals. Three to six measurements were taken from several consecutive slides for each area and each animal. The results were expressed as areas containing immunolabeling within the sample area. These measures were averaged for each area in each animal, and the averages were compared between groups of animals. All data were analyzed by analysis of variance followed by Scheffe post hoc tests. Significance was defined as $P < 0.05$. The data analysis relied on our earlier studies showing an interaction between androgen treatment and WKY and SHR rats [17].

3. Results

3.1. Behavior

Spatial cognition in the Morris water maze was evaluated in WKY and SHR male rats who were exposed to testosterone or cholesterol on postnatal day 10 (Fig. 1). Comparisons were made between the time taken to find the platform as an indicator of spatial memory on the 4 days of testing trials (acquisition period) and final test day (no data was collected on the first day of habituation). The WKY cholesterol control animals exhibited a stepwise decrease in their average time taken to find the platform over the acquisition period and on the final test day. The stepwise pattern in latency to find the platform in WKY controls was similar to the testosterone-treated animals response after the second acquisition day; however, the latter group took longer to attain the shorter latencies. SHR rats administered cholesterol as vehicle control were significantly deficient in finding the platform compared

to WKY cholesterol- or testosterone-treated animals ($P < 0.01$) on the first and last test days (days 2 and 6). SHR testosterone-treated animals were significantly deficient in finding the platform compared to WKY cholesterol- or testosterone-treated animals ($P < 0.001$) and SHR vehicle-treated animals ($P < 0.05$) on the final two test days (days 5 and 6). In fact, while testosterone treatment had no significant effect on WKY by final test day (day 6), testosterone-treated SHR animals were about 30% slower to find the platform than the cholesterol treated controls ($P < 0.05$). Taken together, WKY rats, regardless of treatment, were three to four times faster at finding the platform on the final day than SHR rats (Fig. 1).

3.2. Hormone levels

Basal levels of ACTH and corticosterone were compared between groups (Figs. 2 and 3). Basal levels of ACTH were similar between WKY and SHR control animals. In addition, testosterone treatment had no effect on the pituitary basal tone in WKY animals with no resulting changes in ACTH plasma levels. However, androgen treatment increased basal ACTH plasma levels by about 50% in SHR rats ($P < 0.05$).

Changes were observed in corticosterone levels with testosterone administration in WKY and SHR animals (Fig. 3). Basal levels of corticosterone were elevated in WKY administered vehicle control compared to their SHR counterparts ($P < 0.01$). Similarly, basal levels of corticosterone were elevated in testosterone-treated WKY animals compared to their SHR counterparts ($P < 0.01$). However, no differences were observed within animal strains.

3.3. Catecholamine immunoreactivity

The immunocytochemical procedure led to an optimal labeling of TH-ir fibers throughout the brain. A high density of TH-ir fibers and terminals were observed in the striatum and to a lesser extend in the nucleus accumbens. Fewer fibers were observed in the cortex at the level of the frontal cortex (layers 6 and 1–2). On the peripheral layers, most fibers were oriented parallel to the curvature of the brain. At the level of the junction between the cingulate and the frontal cortex, fibers were seen crossing the different layers, perpendicular to the curvature of the brain (Fig. 4).

The density of TH- ir fibers was compared between testosterone and vehicle administered WKY and SHR animals. Testosterone treatment did not alter the density of TH-ir fibers in the frontal cortex in WKY control animals (Fig. 5a). However, the SHR animals (vehicle controls) showed a significantly lower density

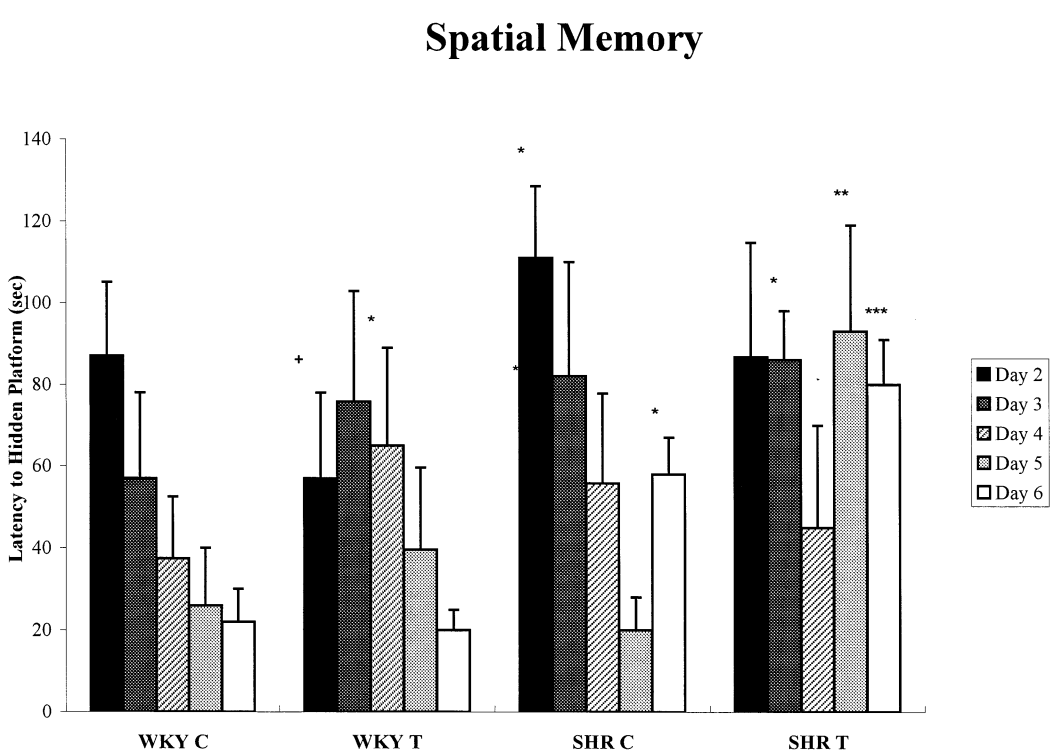

Fig. 1. Spatial memory as measured by the time taken (s) to find the platform in the swim test for WKY and SHR animals treated with cholesterol (C) and testosterone (T) during early postnatal development. Numbers represent average + S.E.M. WKY or SHR animals that took significantly longer to find the platform as compared to WKY treated with cholesterol (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). WKY or SHR animals that found the platform significantly faster than WKY animals treated with cholesterol ($P < 0.05$).

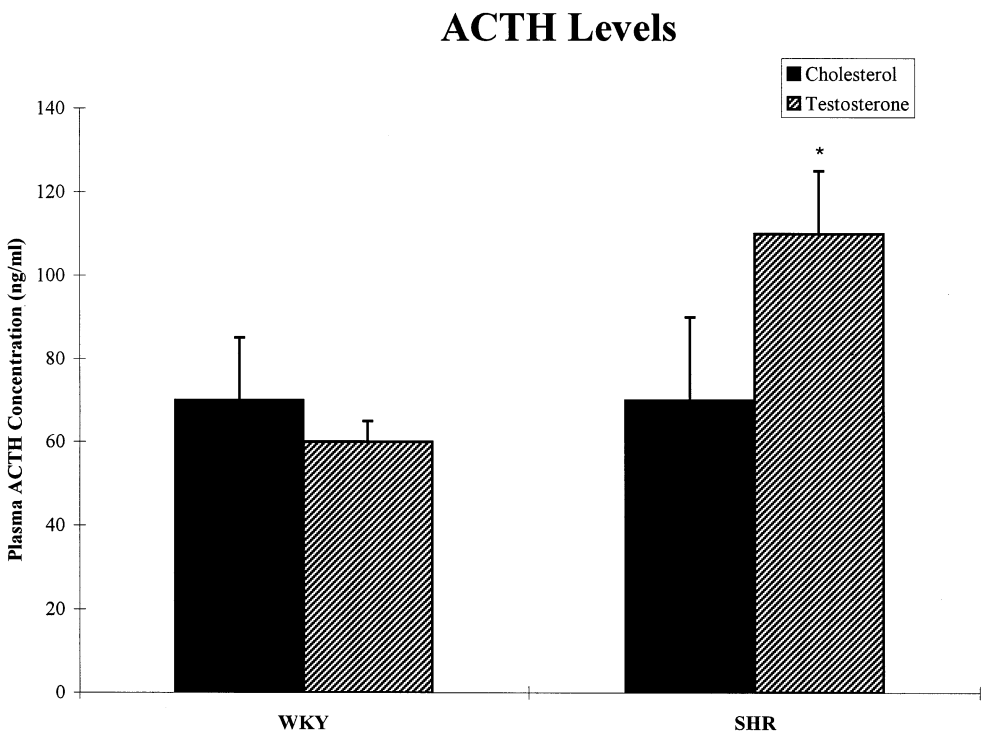

Fig. 2. Baseline ACTH concentration (ng/ml) in adult WKY and SHR animals treated with cholesterol (vehicle) and testosterone during early postnatal development. Numbers represent average + S.E.M. SHR animals treated with testosterone had significantly higher levels of ACTH than WKY animals and SHR animals treated with cholesterol ($*P < 0.05$).

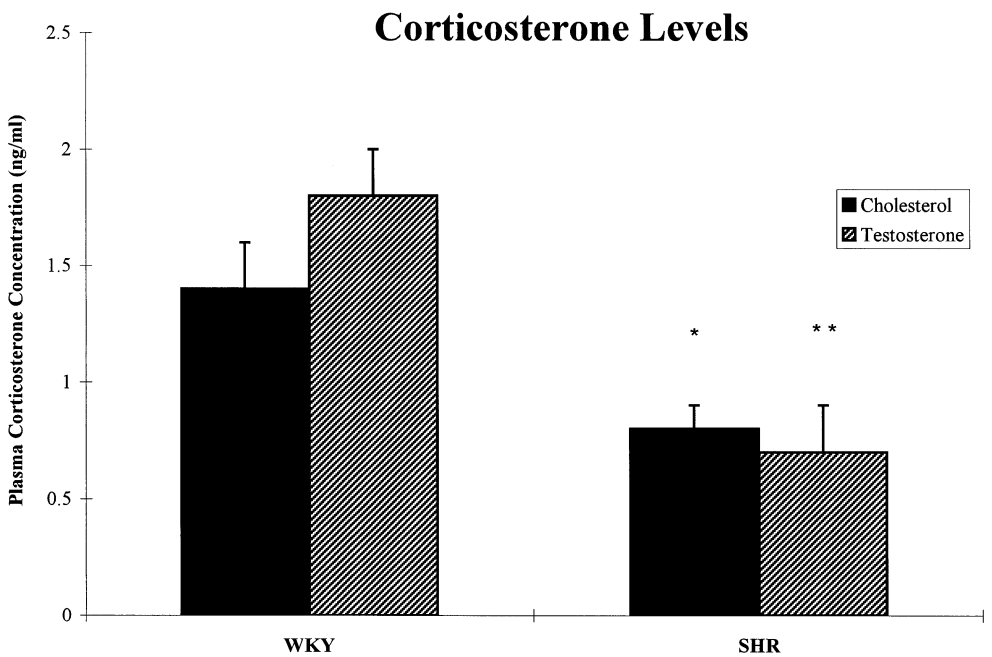

Fig. 3. Baseline corticosterone concentration (ng/ml) in adult WKY and SHR animals treated with cholesterol (vehicle) and testosterone during early postnatal development. Numbers represent average + S.E.M. SHR animals treated with testosterone and cholesterol had significantly lower corticosterone levels than WKY animals treated with testosterone($*P < 0.01$) or cholesterol ($**P < 0.01$).

of TH-ir fibers in this region compared to WKY controls ($P < 0.05$). In addition, SHR animals treated with testosterone had even lower levels of TH-ir in the frontal cortex ($P < 0.01$) compared to WKY testosterone-treated animals and those administered cholesterol vehicle ($P < 0.01$). In contrast, the density of TH-ir in the nucleus accumbens did not differ within strains or between treatments. The comparison of ty-

rosine hydroxylase immunoreactivity lead to similar results between all groups (Fig. 5b).

4. Discussion

The present data support the hypothesis that early in development increased testosterone may lead to changes in cognitive functioning, neuroendocrine parameters, and dopamine innervation in the frontal cortex, in ways that are consistent with ADHD in males. The treatment was timed to reflect approximately the first year of life for humans, whereas the testing was performed to reflect the fifth to seventh year when diagnosis of ADHD generally occurs. The cognitive studies point to inconsistency in the learning to find the platform over time in the ADHD animal model, suggesting deficits in learning (see Fig. 1) that may reflect the inability to focus consistently on the cues provided. These deficits in spatial learning may be augmented in the male SHR rat, which has been shown to exhibit more impulsive behavior than female SHR rats [6]. In addition, treatment with testosterone may further enhance these behaviors.

The characteristic ADHD-like cognitive impairments are accompanied by significant changes in basal levels of hormonal indices of the pituitary–adrenal axis, particularly ACTH and corticosterone. Abnormalities in basal and activated functioning of the endocrine system have been reported in both ADHD children [15,16] and SHR rats [18,39,41]. Our data support the hypothesis that testosterone differentially affects neuroendocrine parameters in SHR animals as compared to controls animals. This particular endocrine milieu (i.e. high ACTH levels) may facilitate further androgen secretion, since it has been shown that ACTH is the primary androgenic stimulator [28]. Although the lower levels of corticosterone seen in ADHD animals are surprising, this may indicate a desensitization of the negative feedback system at the level of the pituitary gland. Dysfunction of this pituitary–adrenal axis has also been reported in children with persistent ADHD, who also have low basal cortisol levels [16]. Such hypothalamic–pituitary–adrenal dysfunctions has been seen in other mental health disorders, such as post-traumatic stress disorder and depression [42], conditions that can be co-morbid with ADHD.

SHR animals showed marked decreases in brain development. These animals are genetically bred animals with many features of ADHD in humans, including overactivity, impaired ability to sustain attention, and impulsivity [9,31]. The immunocytochemical studies demonstrated a lower density of TH-ir in the frontal cortex of the SHR animals compared to WKY controls. This lower density was further suppressed by testosterone treatment (see Figs. 4 and 5), supporting the observation the excess testosterone slows the development of the catecholamine neurons in the frontal cortex of rat pups [36]. In addressing the gender disparities

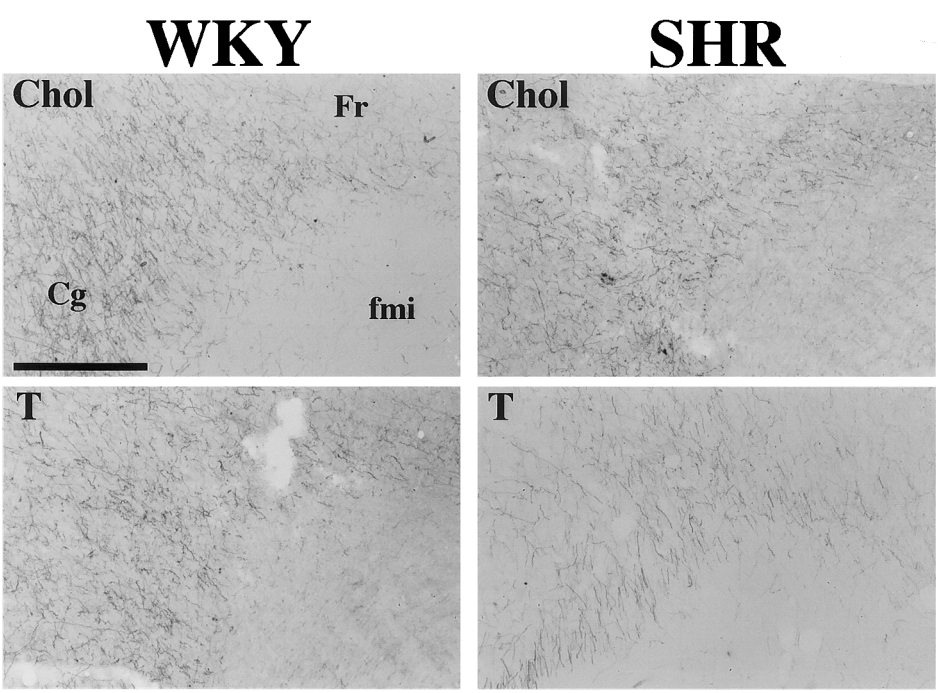

Fig. 4. TH immunoreactivity in the prefrontal cortex of Wistar (control) and SHR (ADHD) animals after testosterone implantation on postnatal day 10. Animals were tested on postnatal day 45. TH immunoreactivity was shown in coronal sections photographed under lightfield. Fr, frontal cortex; Cg, cingulum; fmi, forceps minor corpus callosum; scale bar = 100 μm. Chol, cholesterol (control); T, testosterone. Note the decrease in staining associated with testosterone administration in male ADHD animals.

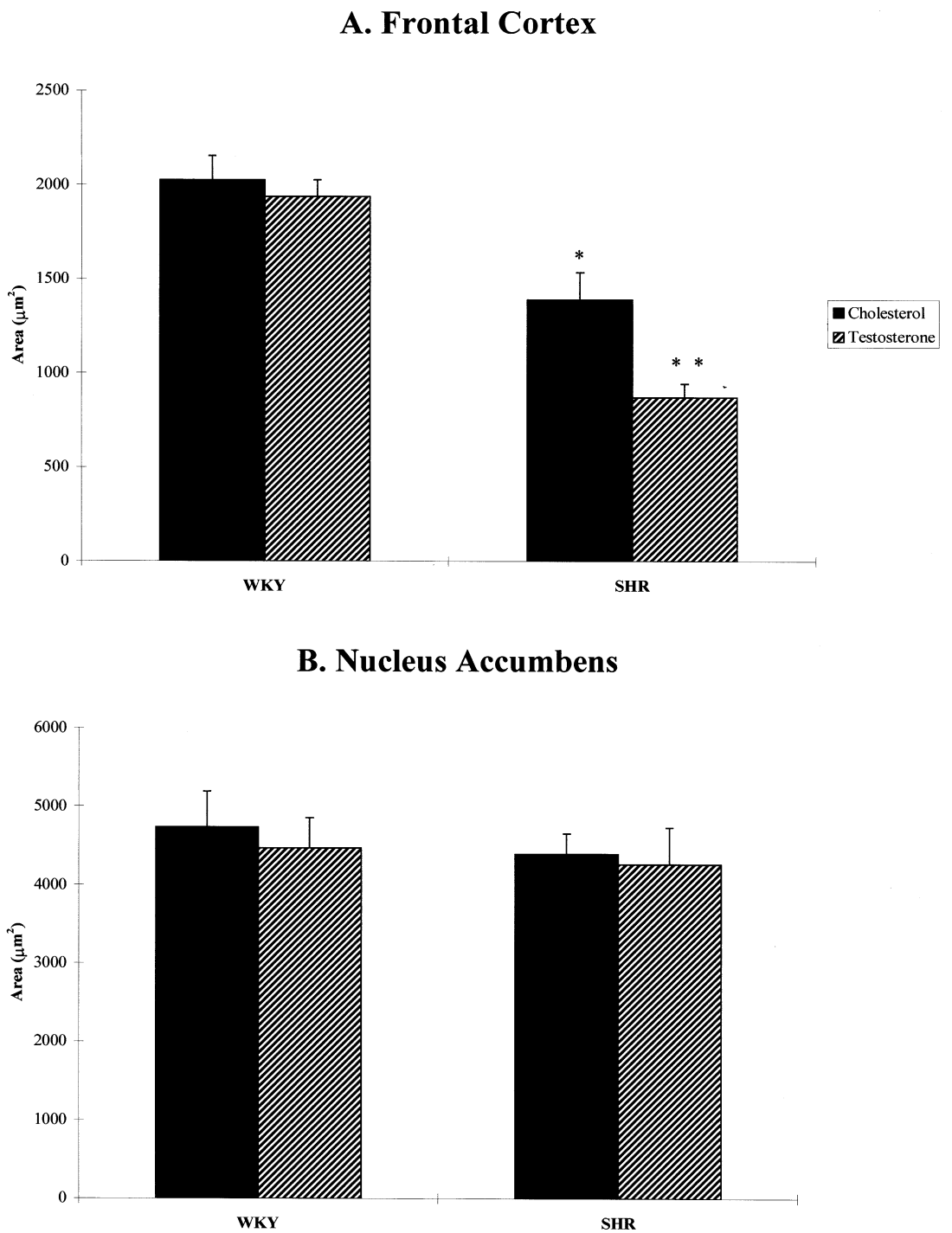

Fig. 5. (A) Comparison of the density of tyrosine hydroxylase immunoreactive fibers, within a sample surface of the frontal cortex of Wistar and SHR animals administered either cholesterol (vehicle) or testosterone during early development. The results are expressed as the area (μm^2) covered by the immunoreactive signal. SHR animals had significantly fewer fibers in this region than Wistar treated with cholesterol ($^*P < 0.05$) or testosterone ($^{**}P < 0.01$). SHR animals treated with testosterone had significantly lower tyrosine hydroxylase immunoreactive fiber density than their cholesterol-treated counterparts ($^{**}P < 0.01$). (B) Comparison of the density of tyrosine hydroxylase-immunoreactive fibers, within a sample surface of the nucleus accumbens of WKY and SHR animals administered either cholesterol (vehicle) or testosterone during early development. The results are expressed as the area (μm^2) covered by the immunoreactive signal. There was no significant difference between groups or treatment.

and underlying neurophysiological basis of ADHD, it maybe likely that the sensitivity difference in adult SHR to testosterone [21] and the significantly higher plasma testosterone [39] strongly suggests that testosterone may play a critical role in predisposing genetically vulnerable male animals to ADHD.

Acknowledgements

We would like to acknowledge the kind technical assistance of Terri-Ann Kelly and Soukaina Adolphe in the preparation of the manuscript.

References

[1] Archer T Jr. Neurotoxin-induced cognitive and motor activity modifications: a catecholamine connection. In: Sagvolden T, Archer T Jr., editors. Attention Deficit Disorder: P Clinical and Basic Research. Hillsdale, NJ: Lawrence Erlbaum, 1989:287–322.

[2] Arnold AP, Breedlove M. Organizational and activational effects of sex steroids on brain and behavior: a reanalysis. Hormones Behav 1985;19:469–98.

[3] Barkley RA. Attention-deficit hyperactivity disorder. Sci Am 1998;279:43–50.

[4] Barkley RA. Attention Deficit Hyperactivity Disorder: a Handbook for Diagnosis and Treatment. New York: Guilford Press, 1990.

[5] Barkley RA, Grodzinsky G, DuPaul GJ. Frontal lobe functions in attention deficit disorder with and without hyperactivity; a review and research report. J Abnorm Child Psychol 1992;20:163–88.

[6] Baum MJ, Brand T, Ooms M, Vreeburg JT, Slob AK. Immediate postnatal rise in whole body androgen content in male rats: correlation with increased testicular content and reduced body clearance of testosterone. Biol Reprod 1998;38:980–6.

[7] Berger DF, Sagvolden T. Sex differences in operant discrimination behaviour in an animal model of attention-deficit hyperactivity disorder. Behav Brain Res 1998;94:73–82.

[8] Bidlingmaier F, Dorr H, Eisenmenger W, Kuhnle U, Knorr D. Contribution of the adrenal gland to the production of androstenedione and testosterone during the first two years of life. J Clin Endocrinol Metab 1986;62:331.

[9] Boix F, Qiao S-W, Kolpus F, Sagvolden T. Chronic L-deprenyl treatment alters brain monoamine levels and reduces impulsiveness in an animal model of attention-deficit hyperactivity disorder. Behav Brain Res 1998;94:153–62.

[10] Forest M, Cathiard A. Pattern of plasma testosterone and androstenedione in normal newborns: evidence for testicular activity at birth. J Clin Endocrinol Metab 1975;41:977.

[11] Forest M, dePeretti E, Bertrand J. Developmental patterns of the plasma levels of testosterone, androstenedione, 17-hydroprogesterone, dehydroepiandrosterone and its sulphate in normal infants and prepubertal children. In: James VHT, Seiro M, Giusti G, Martini L, editors. Endocrine Function of the Human Adrenal Cortex. New York: Academic Press, 1978 561 pp.

[12] Giros B, Jaber M, Jones SR, Wightman RM, Caron MG. Hyperlocomotion and indifference to cocaine amphetamine in mice lacking the dopamine transporter. Nature 1996;379:606–12.

[13] Heilman KM, Voeller KKS, Nadeau SE. A possible pathophysiologic substrate of attention deficit disorder. J Child Neurol 1991;6:S576–81.

[14] Hernandez L, Gonzalez L, Muri E, Paez X, Gottberg E, Baptista T. Testosterone modulates mesolimbic dopaminergic activity in male rats. Neurosci Lett 1994;171:172–4.

[15] Kaneko M, Hoshino Y, Hashimoto S, Okano T, Kumashiro H. Hypothalamic-pituitary-adrenal axis function in children with attention-deficit hyperactivity disorder. J Autism Dev Disord 1993;23:59–65.

[16] King JA, Barkley RA, Barrett S. Attention deficit hyperactivity disorder and the stress response. Biol Psychiatry 1998;44:72–4.

[17] King JA, Ferris CF, Delville Y, Adolphe S, Ray B. Early androgen-induced alterations in stress responsiveness, monoaminergic systems and cognitive performance: possible basis for ADHD. Soc Neurosci 1996;547:15.

[18] Kvetnansky R, McCarty R, Thoa NB, Lake CR, Kopin J. Sympatho-adrenal responses of spontaneously hypertensive rats to immobilization stress. Am J Physiol 1979;236:H457–62.

[19] Lahey BB, Applegate B, McBurnett K, Biederman J, Greenhill L, Hynd GW, Barkley RA, Newcorn J, Jensen P, Richters J, Garfinkel B, Kerdyk L, Frick PJ, Ollendick T, Perez D, Hart EL, Waldman I, Shaffer D. DSM-IV field trials for attention deficit/hyperactivity disorder in children and adolescents. Am J Psychiatry 1994;151:1673–85.

[20] Lunn SF, Recio R, Morris K, Fraser HM. Blockade of the neonatal rise in testosterone by a gonadotrophin-releasing hormone antagonist: effects on timing of puberty and sexual behavior in the male marmoset monkey. J Endocrinol 1994;141:439–47.

[21] Magnusson K, Wall A, Meyerson BJ. Difference in testosterone sensitivity in male spontaneously hypertensive (SHR) and wistar-kyoto rats (WKY). Physiol Behav 1996;60:907–12.

[22] Mook DM, Neuringer A. Different effects of amphetamine on reinforced variations versus repetitions in spontaneously hypertensive rats (SHR). Physiol Behav 1994;56:939–44.

[23] Mack CM, McGivern RF, Hyde LA, Denenberg VH. Absence of postnatal testosterone fails to demasculinize the male rat's corpus callosum. Brain Res Dev Brain Res 1996;95:252–5.

[24] Motelica-Heino I, Castanier M, Corbier P, Edwards DA, Roffi J. Testosterone levels in plasma and testes of neonatal mice. J Steroid Biochem 1988;31:283–6.

[25] Nigg JT, Swanson JM, Hinshaw SP. Covert visual spatial attention in boys with attention deficit hyperactivity disorder: lateral effects, methylphenidate response and results for parents. Neuropsychologia 1997;35:165–76.

[26] Oades RD. Frontal, temporal and lateralized brain function in children with attention -deficit hyperactivity disorder (ADHD): a psychophysiological and neuropsychological viewpoint on development. Behav Brain Res 1998;94:83–95.

[27] Pang SF, Tang F. Sex differences in the serum concentrations of testosterone in mice and hamsters during critical periods of neural sexual differentiation. J Endocrinol 1984;100:7–11.

[28] Parker LN. Control of adrenal androgen secretion. In: Adrenal Androgens in Clinical Medicine. New York: Academic Press, 1989:1989.

[29] Reisert I, Han V, Lieth E, Toran-Allerand D, Pilgrim C, Lauder J. Sex steroids promote neurite growth in mesencephalic tyrosine hydroxylase immunoreactive neurons in vitro. Int J Dev Neurosci 1987;5:91–8.

[30] Sagvolden T, Metzger MA, Schiorbeck HK, Rugland AL, Spinnagr I, Sagvolden G. The spontaneously hypertensive rat (SHR) as an animal model of childhood hyperactivity (ADHD): changed reactivity to reinforcers and to psychomotor stimulants. Behav Neural Biol 1992;58:13–112.

[31] Sagvolden T, Pettersen MB, Larsen MC. Spontaneously hypertensive rats(SHR) as a putative animal model of childhood hyperkinesis: SHR behavior compared to four other rat strains. Physiol Behav 1993;54:1047–55.

[32] Sagvolden T, Aase H, Zeiner P, Berger DF. Altered reinforcement mechanisms in attention-deficit hyperactivity disorder. Behav Brain Res 1998;94:61–71.

[33] Shaywitz MBA, Klopper JH, Yager RD, Gordon JW. Paradoxical response to amphetamine in developing rats treated with 6-hydroxydopamine. Nature 1976;261:153–5.

[34] Shipley MT, Luna J, McLean JH. Processing and analysis of neuroanatomical images. In: Heimer L, Zaborsky L, editors. Neuroanatomical Tract Tracing Methods II. New York: Plenum, 1989:331–90.

[35] Slob AK, Ooms MP, Kreeburg JTM. Prenatal and early postnatal sex differences in plasma and gonadal testosterone and plasma luteinizing hormone in female and male rats. J Endocrinol 1980;87:81–7.
[36] Stewart J, Rajabi H. Estradiol derived from testosterone in prenatal life affects the development of catecholamine systems in the frontal cortex in male rats. Brain Res 1994;646:157–60.
[37] Szatmari P, Boyle MN, Offord DR. ADHD and conduct disorder: degree of diagnostic overlap and differences among correlates. J Am Acad Child Adolesc Psychiatry 1989;28:865–72.
[38] Taylor E. Clinical foundation of hyperactivity research. Behav Brain Res 1998;94:11–24.
[39] Wall A, Magnusson AM, Meyerson BJ. The influences of androgen on sociosexual behavior: a comparison between the spontaneously hypertensive (SHR) and the Wistar-Kyoto rat (WKY). Physiol Behav 1993;54:1041–6.
[40] Watson RE, Weigand SJ, Clough JA, Hoffman GE. Use of cryoprotectant to maintain long-term peptide immunoreactivity and tissue morphology. Peptides 1986;7:155–9.
[41] Yamamoto J, Nakai M, Natsume T. Cardiovascular responses to acute stress in young-to-old spontaneously hypertensive rats. Hypertension 1987;9:362–70.
[42] Yehuda R, Teicher MH, Trestman RL, Levengood RA, Siever LJ. Cortisol regulation in posttraumatic stress disorder and major depression: a chronobiological analysis. Biol Psychiatry 1996;40:79–88.