Central aromatization of testosterone in testicular feminized mice[1]

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Summary. Aromatization of testosterone was examined in hypothalamic and cerebral cortex tissues from 32 mice – 10 normal males, 10 normal females, 2 carrying the testicular feminized gene (Tfm) and 10 Tfm with the modifying (o^{hv}) gene. Total aromatization was 1.5 times greater in normal males than females. In both forms of Tfm, conversions were equal and similar to normal females.

Aromatization in the mammalian brain was first suggested by Knapstein et al.[3], and subsequently confirmed by other workers[4–7]. It had also been proposed that aromatization in the hypothalamus may be responsible for imprinting the brain and inhibiting the development of cyclicity in rats[4]. Neonatal androgen secretion therefore serves to masculinize the male rodent with subsequent development of male behavioural and neuroendocrinological patterns[8,9].

Animals carrying the testicular feminization (Tfm) mutation which is X-linked in all animals[10] including man[11] are unresponsive to testosterone. It was recently shown that a variant Tfm resulting from a change in the nearby 'controlling element'[12], modifies the expression of the Tfm mutation such that some male characteristics develop[13]. This variant was designated Tfm (o^{hv}). Our working hypothesis has therefore been that if androgens organize the hypothalamus in a male direction then because of androgen insensitivity Tfm mice might be expected to be different from normal mice.

Mice carrying the Tfm (2) and modifying (o^{hv}) gene (10) (maintained at the City of Hope National Medical Centre), as well as normal BALB/c males (10) and females (10) (Health Research Inc.), were approximately 30 days old at the time of the experiments. Except for the Tfm the other 3 groups were each subdivided into 2 groups of 5 each. Mice were killed by cervical dislocation and sections of hypothalamus and cerebral cortex, approximately 2 mm^3 removed and placed in ice-cold isotonic saline. Combined tissue was minced with scissors, blotted dry and weighed.

Incubations were carried out in 10 ml Erlenmeyer flasks containing 5 μCi 4-^{14}C-testosterone (58.8 mCi/mmole, New England Nuclear), 10 μmoles nicotinamide adenine dinucleotide phosphate, 10 μmoles adenosine triphosphate, 60 μmoles glucose 6-phosphate and 20 units glucose 6-phosphate dehydrogenase in 1ml Hank's balanced salt solution, pH 7.4. Control incubations contained no tissue. Incubations were performed in a Dubnoff metabolic shaker at 37 °C for 3 h with O_2:CO_2::95:5 as the gas phase. At the end of the incubations the media were quick-frozen and stored at −78 °C until analysis.

After thawing, 50 μg of carrier and 20,000 dpm tritium labelled estrone, estradiol-17β and estriol were added to each incubation flask prior to extraction with diethyl ether. The combined ether extracts were evaporated to dryness and subjected to toluene 1 N NaOH partition. The sodium hydroxide layer was subjected to extractive alkylation as previously described[14]. Recoveries from the incubates using this novel methylation procedure was 81.5 ± 2.1%. After chromatography on a florisil column the methylated extract was subjected to thin layer chromatography (silica gel F-254, system benzene:ethyl acetate::2:1). Radioactive areas were identified by autoradiography. 3 major areas were found with R_f 0.51 (estrone methyl ether), R_f 0.37 (unknown) and R_f 0.32 (estradiol methyl ether). Carrier steroids were added to the appropriate eluates from the thin layer plates and crystallized to constant specific activity. Conversions were calculated from the final specific activity of the crystals and expressed as a percentage per 100 mg tissue, corrected for losses.

The percentage conversions of 4-^{14}C-testosterone to estrogens by hypothalamus and cerebral cortex tissue from normal BALB/C and Tfm mice are shown in the table. Conversions were higher in hypothalamus than in cortex. Hypothalamus of males showed higher conversions than females and both Tfm mutants had conversions similar to females.

Aromatization of 4-^{14}C-testosterone by hypothalamus and cortex of mice

Tissue	Genotype	% conversion/100 mg tissue/3 h Estrone	Estradiol	Total
Hypothalamus	BALB/C (M)*	0.12	0.04	0.16
	BALB/C (F)	0.09	0.02	0.11
	Tfm (o^+)	0.03	0.09	0.12
	Tfm (o^{hv})	n.d.	0.11	0.11
Cortex	BALB/C (M)	0.03	0.01	0.04
	BALB/C (F)	0.02	0.01	0.03
	Tfm (o^+)	n.d.	0.06	0.06
	Tfm (o^{hv})	n.d.	0.04	0.04

*M = male, F = female. Tfm (o^+) = Normal testicular feminized mice; Tfm (o^{hv}) = testicular feminized mice with modifying gene. n.d. = Not detectable. Results are mean conversions.

1 This work was supported by the Medical Research Council of Canada.

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The demonstration that the cerebral cortex of all mice is capable of some aromatization is noteworthy since other workers have failed to find such evidence in other rodents or mammals[4,5,7]. This inability to show aromatization may be due to low recoveries and/or age dependent changes in enzyme activity. That cortex can concentrate radiolabel from administered androgens has been demonstrated by several groups[15–18]. However, these authors did not determine whether any of the radioactivity concentrated in this brain region was derived from aromatization of accumulated androgen. Human fetal cortex has been shown to aromatize androgens[5] which is comparable to our observations on 30-day-old mouse cortex. In the present study no sex differences could be detected in cerebral cortex aromatization.

Mouse hypothalamic tissue[5] has been reported to aromatize androstenedione with a male:female ratio of 3. Since neither age of mice nor percentage conversions were given it was not possible to make direct comparisons with our results. Moreover, it was suggested that Tfm mice had aromatizing ability similar to that of normal males[5]. This is in contrast to the present data where the ability of both mutants was towards the female direction, and are consistent with data using liver tissue where it was demonstrated that in androgen insensitive rats steroid metabolism proceeds along female lines[19].

It is also of interest that there was a good conversion of testosterone to estrone in hypothalamic tissue of normal mice whereas in Tfm the major estrogen formed was estradiol-17β. Studies on further metabolism of testosterone in central tissues of genetic mutant mice are still in progress.

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Effects of handling normal and bulbectomized rats at adrenal and plasma corticosterone levels

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Summary. Normal and bulbectomized adult rats, male and female, handled daily for 4 weeks, show plasma and adrenal corticosterone values significantly lower than the non-handled ones.

In our previous investigations, we were able to study the effects of removal of olfactory bulbs on the corticoadrenal function in female rats[1,2]. The animals were handled during a predetermined period in order to avoid stress at the moment of decapitation. Bearing in mind that the activity of adrenal glands can be influenced by many factors, we wondered whether daily handling, in itself, would affect adrenocortical secretion. If this were the case, would it reveal itself in a similar manner in both normal and bulbectomized rats? The present study was undertaken to clarify this point.

Material and methods. 67 white adult rats bred in our institute were used: 34 females of between 160 and 270 g weight, and 33 males of between 230 and 360 g weight. All animals were kept under the same conditions, housed 8 per cage, with food and water available ad libitum. Animals of each sex were divided into 4 lots: a) non-handled normal rats; b) handled normal rats; c) non-handled rats with bilateral removal of olfactory bulbs; d) handled rats with bilateral removal of olfactory bulbs. Handling consisted in taking rats one at a time and holding them for a few seconds outside the cage, twice a day, for 5 days in the week. The cages of non-handled rats were only opened twice a day. After 4 weeks' handling, the rats were decapitated by means of a small animal guillotine at between 9 and 11 a.m., the time between

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	Corticosterone			
	Adrenal (μg/g)		Plasma (μg/100 ml)	
	Non-handled	Handled	Non-handled	Handled
Female				
Normal	20.9 ± 5.20* (8)	7.2 ± 1.19 (8)	35.1 ± 4.98 (8)	14.1 ± 1.26 (8)
Bulbectomized	10.0 ± 2.30 (7)	3.7 ± 0.55 (11)	19.9 ± 1.97 (7)	6.9 ± 1.10 (10)
Male				
Normal	10.5 ± 2.43 (9)	3.8 ± 0.71 (8)	19.8 ± 3.70 (9)	9.0 ± 1.48 (8)
Bulbectomized	11.2 ± 2.18 (9)	3.9 ± 0.59 (7)	20.2 ± 3.13 (9)	7.3 ± 2.07 (7)

*Mean ± SE. Number of animals is given in parentheses.