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# The sex difference of plasma homovanillic acid is unaffected by cross-sex hormone administration in transsexual subjects

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## Abstract

There is a close relationship between the brain and the endocrine system. The brain expresses receptors for sex steroids and is capable of metabolizing these hormones. We explored (1) sex differences in homovanillic acid (HVA), a metabolite of the neurotransmitter dopamine, and (2) the effects of cross-sex steroid administration in transsexual subjects. First, we compared plasma HVA levels between 38 male and 34 female healthy volunteers (not using hormone replacement therapy) of a mean age of 72 years (range 65–84 years). Secondly, we measured plasma HVA levels in 15 male-to-female transsexuals treated with 100 µg ethinyl estradiol/day and 100 mg cyproterone acetate/day for 4 months, and in 17 female-to-male transsexuals treated with testosterone esters (250 mg/2 weeks i.m. for 4 months). Plasma HVA levels were lower in elderly men than in elderly postmenopausal women (geometric mean 25.4 nmol/l (percentile (P)<sub>10</sub> 4.9; P<sub>90</sub> 69.8) vs 39.0 nmol/l (19.0; 76.1);  $P=0.027$ ). In

transsexuals before cross-sex hormone administration, genetic males also had lower plasma levels of HVA than genetic females (geometric mean 14.8 nmol/l (P<sub>10</sub> 7.0; P<sub>90</sub> 35.0) vs 34.3 nmol/l (21.8; 61.4);  $P<0.001$ ). Cross-sex hormone administration did not affect plasma HVA in either group ( $P>0.5$ ). The pretreatment sex difference in plasma HVA was unaffected after 4 months of cross-sex hormone administration ( $P=0.003$ ). The sex difference in plasma HVA was not reversed by cross-sex hormone administration in transsexuals, and was also preserved in elderly subjects. This indicated that differences in dopamine gene expression were largely unaffected by exposure to sex hormone levels in adulthood, but must rather be explained by a sex difference in genetic factors or by the organizing effects of sex hormones during early development.

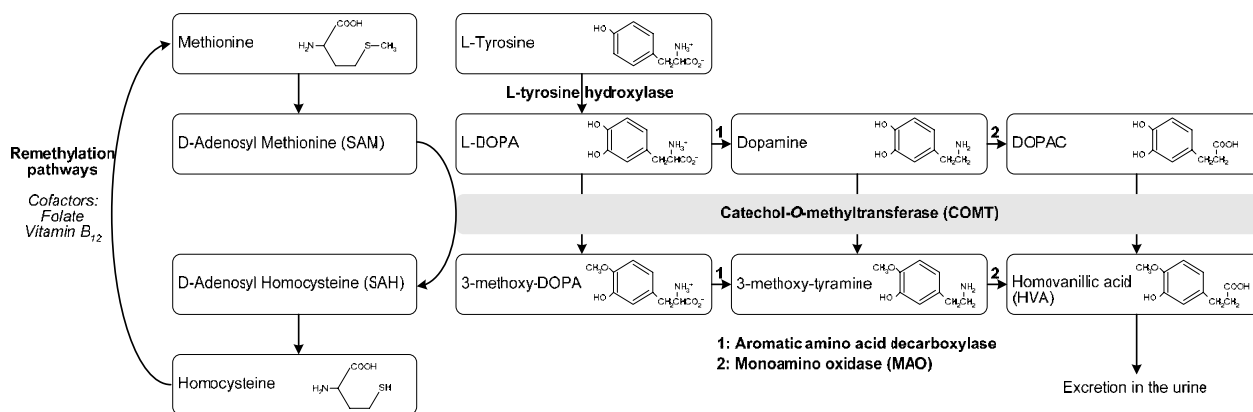
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## Introduction

Sex differences in schizophrenia, with an earlier onset (Hambrecht *et al.* 1992, Albus & Maier 1995, Szymanski *et al.* 1995, Halbreich & Kahn 2003) and a higher prevalence (Aleman *et al.* 2003) in men than in women, may indicate that sex hormones are implicated in its expression, etiology and course. The brain contains receptors (Kruijver *et al.* 2001, 2003, Toran-Allerand 2004) for androgens and estrogens, and is capable of synthesizing and metabolizing these steroids (Puy *et al.* 1995, Poletti & Martini 1999, Stoffel-Wagner 2003, Altman 2004). Dopamine is synthesized from tyrosine by dopaminergic neurons, and is also the precursor to norepinephrine in noradrenergic nerves. Dopamine is metabolized in the brain, liver and kidney to its inactive metabolite homovanillic acid (HVA; Fig. 1), and in human (Amin *et al.*

1995, Amin *et al.* 1998) and animal (Bacopoulos *et al.* 1979) research its plasma concentration was found to be indicative of brain dopamine turnover. Plasma HVA is not only elevated in neuroblastomas and pheochromocytomas, but also in schizotypal personality disorders (Siever *et al.* 1991). In some (Pickar *et al.* 1986, Davidson & Davis 1988, Maas *et al.* 1988, Davidson *et al.* 1991, Koreen *et al.* 1994, Sumiyoshi *et al.* 1997a) but not all (Sumiyoshi *et al.* 1997b) studies, the baseline and/or change in plasma HVA was related to psychopathology and treatment response in schizophrenic patients.

Sex steroids have been found to affect neurotransmitters and neurotransmitter receptors of the dopaminergic systems in complex ways, at least in cell and animal studies (Becker 1999, Eaton *et al.* 1999, Andersen *et al.* 2002, Landry *et al.* 2002, Halbreich & Kahn 2003, D'Astous *et al.* 2004). In humans, the potential effects of sex



**Figure 1** Role of catechol-O-methyltransferase (COMT)-mediated methylation in dopamine metabolism. The major detoxification pathway (i.e. metabolizing dopamine to inactivate metabolites) involves *O*-methylation at the meta-hydroxyl group and oxidative deamination. *O*-Methylation is catalyzed by the enzyme COMT and oxidative deamination is catalyzed by MOA, primarily MAO B. HVA is the end product of dopamine metabolism and can be used as a marker of dopamine turnover. Methionine forms *S*-adenosylmethionine (SAM), which serves as the methyl donor to dopamine (and other compounds). Homocysteine, which is formed during this metabolism of methionine, is metabolized back into methionine through one of two remethylation pathways, one of which using folate as a cofactor. DOPA, 3,4-dihydroxyphenylalanine; DOPAC, 3,4-dihydroxyphenylacetic acid.

hormone administration on dopaminergic activity have not yet been studied extensively (Lobo *et al.* 1984, Hannan *et al.* 1991, Paoletti *et al.* 2001). Administration of testosterone, which endogenously is partially aromatized to estrogens, did not affect serum HVA in a small group of men (Hannan *et al.* 1991), although nandrolone decanoate, only a weakly aromatizable synthetic anabolic steroid, did increase serum HVA. A consistent sex difference has been described for plasma HVA levels. Higher plasma levels of HVA are found in female patients with schizophrenia (Koreen *et al.* 1994, Davila *et al.* 1995, Szymanski *et al.* 1995, Sumiyoshi *et al.* 1997b), in female patients with psychotic disorders (Bowers *et al.* 1983, Bowers & Swigar 1987), in female patients with tardive dyskinesia (Glazer *et al.* 1983), as well as in healthy female controls (Koreen *et al.* 1994, Sumiyoshi *et al.* 1997b) as compared with their male counterparts. This sex difference in plasma HVA implies sex differences in dopamine metabolism but not necessarily in dopamine levels.

Studies in transsexual subjects have shown that cross-sex hormones affect psychological variables of aggression, mood, sexual motivation and sex-related cognitive functions, such as verbal fluency and visuospatial functions (Van Goozen *et al.* 1995, Slabbekoorn *et al.* 1999). Studies of the brains of transsexuals have shown that certain sex dimorph areas of the brain have a differentiation of the opposite sex (female in male-to-female transsexuals and vice versa). These anatomical brain structures were not affected by the administration of cross-sex hormones (Zhou *et al.* 1995, Kruijver *et al.* 2000). In the present study, we have investigated whether changes in sex hormones affect the sex difference in plasma HVA, to test the hypothesis as to whether human dopamine metabolism is under sex steroidal influence. Blood samples were

collected as part of a study on the effects of sex hormones on cardiovascular risk factors (Giltay *et al.* 2000, 2003). We measured fasting plasma HVA levels because of the better reproducibility and smaller variability as compared with urinary HVA (Donnelly *et al.* 1996, Amin *et al.* 1998). It is also a less invasive approach than sequentially obtaining cerebrospinal fluid.

## Materials and Methods

First, the sex difference in HVA and 5-hydroxyindoleacetic acid (5-HIAA) were studied in 38 male and 34 female healthy volunteers of the Arnhem Elderly Study, a population-based cohort study that started in 1991 (Giltay *et al.* 2004). In total, 641 independently living men and women aged 65–84 years in the city of Arnhem agreed to have a venipuncture. Plasma HVA and 5-HIAA was assessed in a random sample of 72 subjects. None of these postmenopausal women were using hormone replacement therapy. The body mass index (BMI; weight/height<sup>2</sup>) was assessed. All subjects provided written informed consent. The study was approved by the ethical committee of Wageningen University.

Secondly, the effects of cross-sex hormone administration on HVA and 5-HIAA were studied in transsexual subjects in blood samples collected from 1996 to 1998 (Giltay *et al.* 2000, 2003). Psychological criteria for the diagnosis and treatment followed the guidelines provided by the Harry Benjamin International Gender Dysphoria Association. Fifteen male-to-female (M→F) transsexuals were treated with ethinyl estradiol (Lynoral; 100 µg/day; Organon, Oss, The Netherlands) in combination with cyproterone acetate (Androcur; 100 mg/day; Schering,

Berlin, Germany), an anti-androgen that blocks androgen receptors. Seventeen female-to-male (F→M) transsexuals were treated with testosterone esters (Sustanon; 250 mg/2 weeks i.m.; Organon) according to our standard treatment. To monitor the anabolic/catabolic effects on body composition, the BMI was assessed, and lean body mass and total body fat were estimated using bioelectrical impedance analysis (BIA 101/S; RJL Systems, Clinton Twp, MI, USA). Informed consent was obtained from all subjects, and the study was conducted according to the principles of the Declaration of Helsinki and approved by the Ethical Review Committee of the Vrije Universiteit University Medical Center.

#### Laboratory tests

A single non-fasting blood sample was first obtained from elderly subjects. Venipuncture was performed between 0800 and 1730 h using citrate collection tubes. Samples were stored at  $-80^{\circ}\text{C}$ , and only thawed once before analysis. Secondly, in transsexual subjects, blood was sampled before and 2 and 4 months after initiation of cross-sex hormone administration. Blood was drawn from genetic women before testosterone treatment between days 5 and 9 of the follicular phase and again during testosterone treatment, within 5 to 9 days after the testosterone injection. All blood was sampled after a 12-h fast, immediately placed on ice, centrifuged at 3500 g for 30 min at  $4^{\circ}\text{C}$ , stored within 1 h at  $-80^{\circ}\text{C}$  and never thawed until analysis. EDTA plasma samples (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) were obtained. Serum measurements were made of  $17\beta$ -estradiol (by radioimmunoassay; Sorin Biomedica, Saluggia, Italy) and testosterone (by radioimmunoassay; Coat-A-Count; Diagnostic Products, Los Angeles, CA, USA), luteinizing hormone (LH; by immunometric luminescence assay; Amerlite; Amersham Pharmacia Biotech, Aylesbury, Bucks, UK) and follicle-stimulating hormone (FSH; by immunometric luminescence assay; Amerlite). Since estrogens increase the secretion of prolactin by the pituitary gland and prolactin release is tonically inhibited by dopamine, we measured prolactin levels (by immunoradiometric assay; Biosource Technologies, Inc., Fleurus, Belgium). HVA and 5-HIAA are cleared by the kidney so we therefore assessed creatinine levels by using standard laboratory methods. Because HVA is formed in close conjunction with homocysteine synthesis (Fig. 1), we measured plasma levels of total homocysteine and folate by high-performance liquid chromatography (HPLC) as previously published (Giltay *et al.* 2003). Moreover, folate may also be indirectly involved in rate-limiting enzymes for dopamine and serotonin production through tetrahydrobiopterin (Bottiglieri *et al.* 2000).

To measure HVA and 5-HIAA, the major metabolite of serotonin, samples were mixed with an equal volume of perchloric acid (1 M) in order to precipitate proteins. After

centrifugation, the supernatant (250  $\mu\text{l}$ ) was loaded on a Hypersil ODS HPLC column (Thermo Electron Corporation, Breda, The Netherlands) at  $30^{\circ}\text{C}$ . The mobile phase consisted of ammonium acetate (0.02 M), sodium-EDTA (0.25 mM), sodium chloride (6.5 mM) and methanol (9.5%) at pH 4.95. The flow was set at 1.25 ml/min by using an isocratic Sp8810 pump (Thermo Electron Corporation). Detection was performed by using an electrochemical detector (INTRO; Antec Leyden, Zoeterwoude, The Netherlands; potential: 0.525 mV). Chromatographic data were recorded by using PC1000 software (Thermo Electron Corporation). The intra-assay coefficient of variation (C.V.) for HVA was 2.0% at a concentration of 156 nM ( $n=9$ ) and interassay C.V. was 2.7% at a concentration of 160 nM ( $n=7$ ). The intra-assay C.V. for 5-HIAA was 3.8% at a concentration of 93 nM ( $n=9$ ) and the interassay C.V. was 5.5% at a concentration of 55 nM ( $n=7$ ).

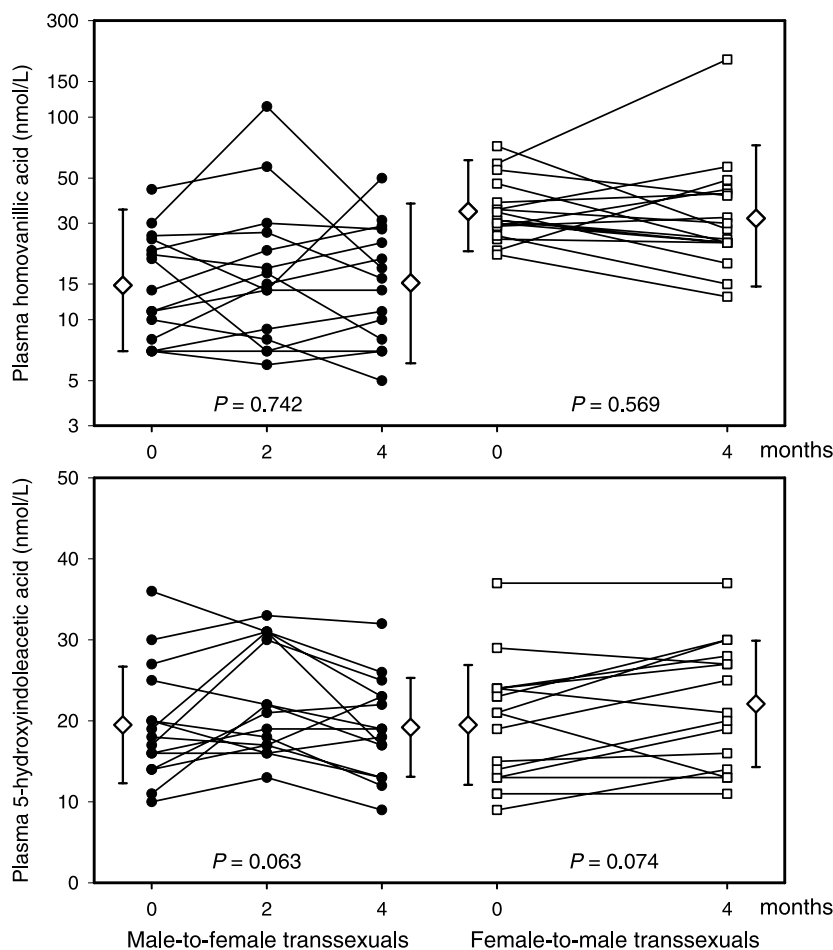
#### Statistical analysis

Data are presented as means (with s.d.) or geometric means (with percentiles:  $P_{10}$  and  $P_{90}$ ) for right-skewed data (i.e. total homocysteine, folate and HVA). HVA and 5-HIAA values were correlated with other variables using Spearman's correlation coefficient. For the comparison at baseline between the two groups, a *t*-test for independent samples or analysis of covariance was used. For M→F transsexuals, a two-factor repeated measures ANOVA with interaction was used to study the effects of estrogens plus anti-androgens in time, as well as to compare the effects of the two treatment regimens in time. Post-hoc comparisons were made using a Sidak test to identify significant differences between time-points. For F→M transsexuals, a Student's *t*-test for paired samples was used to study the effects of androgen administration in time. A two-tailed  $P<0.05$  was considered statistically significant. The software used was SPSS 10.0, Chicago, IL, USA.

## Results

#### Comparison between elderly men and women

Men were, on average, 72.5 years old (s.d. 4.9; range 65–84) and women were 72.8 years old (s.d. 5.8; range 65–83; for sex difference  $P=0.79$ ). The BMI was a mean of 24.6 (s.d. 3.0) in men and 26.1 (s.d. 5.0) in women (for sex difference  $P=0.15$ ). Plasma HVA levels were lower in elderly men than postmenopausal women (geometric mean 25.4 nmol/l ( $P_{10}$  4.9;  $P_{90}$  69.8) vs 39.0 nmol/l (19.0; 76.1);  $P=0.027$ ). Plasma HVA was positively associated with age ( $r=0.26$ ,  $P=0.026$ ). The sex difference for plasma HVA remained present after adjusting for age ( $P=0.033$ ). Plasma 5-HIAA levels were similar in elderly men and women ( $P=0.42$ ), also when one outlier (323 nmol/l) was excluded from the analysis (21.6 nmol/l (s.d. 15.3) vs 21.9 nmol/l (s.d. 24.6);  $P=0.94$ ).



**Figure 2** Individual values of HVA on a logarithmic scale and 5-HIAA in 15 M→F transsexuals (●) at baseline and after 2 and 4 months of administration of ethinyl estradiol plus cyproterone acetate and 17 F→M transsexuals (□) at baseline and after 4 months of administration of testosterone esters. Back-transformed mean values (with confidence intervals) at 0 and 4 months are identified by diamonds. *P* values were assessed by ANOVA for repeated measurements for M→F transsexuals and by *t*-test for paired samples for F→M transsexuals. At baseline, sex differences were statistically significant for plasma HVA ( $P < 0.0005$ ) and not for 5-HIAA ( $P = 0.981$ ). At 4 months, the sex difference remained statistically significant for plasma HVA ( $P = 0.003$ ).

#### Intervention studies with sex steroids

**Pretreatment values** The mean age of M→F transsexuals was 32.4 (s.d. 6.6; range 20–43) years with a BMI of 22.8 (s.d. 2.7) kg/m<sup>2</sup> and that of F→M transsexuals was 27.1 (s.d. 6.3; range 18–37) years with a BMI of 23.9 (s.d. 4.3) kg/m<sup>2</sup>. Of the M→F transsexuals, nine (60%) were smokers and of the F→M transsexuals ten (59%) were smokers.

In M→F transsexuals, baseline HVA levels were inversely associated with testosterone levels ( $r = -0.61$ ,  $P = 0.017$ ) and LH levels ( $r = -0.70$ ,  $P = 0.003$ ), whereas baseline 5-HIAA levels were positively associated with creatinine levels ( $r = 0.53$ ,  $P = 0.050$ ). Smokers had significantly lower HVA levels as compared with

non-smokers ( $P = 0.003$ ), and similar levels of 5-HIAA ( $P = 0.18$ ). In F→M transsexuals, baseline HVA and 5-HIAA levels were not correlated with levels of hormones, creatinine or folate, and baseline HVA levels were not correlated with levels of total homocysteine. Smokers had similar levels of HVA and 5-HIAA as compared with non-smokers ( $P = 0.50$  and  $P = 0.54$  respectively). Age was unrelated to plasma HVA or 5-HIAA in either group ( $P > 0.20$ ).

At baseline, plasma HVA was significantly lower in genetic men as compared with women (geometric mean 14.8 nmol/l ( $P_{10}$  7.0;  $P_{90}$  35.0) vs 34.3 ( $P_{10}$  21.8;  $P_{90}$  61.4);  $P < 0.0005$ ; Fig. 2 and Table 1). The sex difference remained present ( $P = 0.003$ ) after adjusting for age,

**Table 1** Hormone and metabolic data before and after 2 and 4 months in 15 M→F transsexuals randomized for administration of ethinyl estradiol plus cyproterone acetate and in 17 F→M transsexuals treated with intramuscular testosterone esters. Data are means  $\pm$  s.d. or geometric means ( $P_{10}$  and  $P_{90}$ ) for right-skewed data (i.e. total homocysteine, folate and HVA)

	Oral ethinyl estradiol and cyproterone acetate in M→F transsexuals			Intramuscular testosterone esters in F→M transsexuals		
	Baseline	2 months	4 months	Baseline	4 months	$P$ value <sup>1</sup>
Serum testosterone (nmol/l)	22.9 $\pm$ 6.3 <sup>a</sup>	1.1 $\pm$ 0.1 <sup>b</sup>	1.0 $\pm$ 0.1 <sup>b</sup>	2.0 $\pm$ 0.8	33.0 $\pm$ 9.1	<0.0005
Serum 17 $\beta$ -estradiol (pmol/l)	97 $\pm$ 31	— <sup>2</sup>	— <sup>2</sup>	189 $\pm$ 90	127 $\pm$ 36	0.011
Serum LH (IU/l)	3.2 $\pm$ 1.6 <sup>a</sup>	0.3 $\pm$ 0.0 <sup>b</sup>	0.3 $\pm$ 0.0 <sup>b</sup>	5.6 $\pm$ 3.4	2.4 $\pm$ 2.1	0.005
Serum FSH (IU/l)	3.4 $\pm$ 2.0 <sup>a</sup>	0.5 $\pm$ 0.0 <sup>b</sup>	0.5 $\pm$ 0.0 <sup>b</sup>	4.3 $\pm$ 1.0	2.8 $\pm$ 1.1	0.002
Serum prolactin (IU/l)	0.13 $\pm$ 0.03	—	0.44 $\pm$ 0.17	0.25 $\pm$ 0.13	0.31 $\pm$ 0.14	0.114
BMI (kg/m <sup>2</sup> )	22.8 $\pm$ 2.7	—	23.2 $\pm$ 1.8	23.9 $\pm$ 4.3	25.2 $\pm$ 4.3	<0.0005
Total body fat (kg)	12.2 $\pm$ 3.7	—	14.1 $\pm$ 3.4	19.6 $\pm$ 6.6	19.1 $\pm$ 6.8	0.429
Lean body mass (kg)	62.4 $\pm$ 7.8	—	61.7 $\pm$ 5.9	46.7 $\pm$ 6.2	50.9 $\pm$ 7.2	<0.0005
Plasma creatinine ( $\mu$ mol/l)	90.4 $\pm$ 11.4	90.9 $\pm$ 8.1	87.2 $\pm$ 7.4	74.3 $\pm$ 9.5	81.5 $\pm$ 9.1	<0.0005
Plasma total homocysteine ( $\mu$ mol/l)	10.9 (6.4; 25.6) <sup>a</sup>	7.8 (4.9; 15.7) <sup>b</sup>	7.6 (5.1; 12.5) <sup>b</sup>	10.1 (6.4; 17.7)	11.9 (7.3; 27.7)	0.004
Plasma folate (nmol/l)	2.8 (1.7; 5.0)	2.5 (1.1; 5.2)	2.9 (1.1; 8.6)	5.7 (2.0; 10.6)	5.2 (1.8; 10.5)	0.108
Plasma HVA (nmol/l)	14.8 (7.0; 35.0)	16.7 (6.6; 74.9)	15.2 (6.1; 37.5)	34.3 (21.8; 61.4)	31.7 (14.6; 72.7)	0.569
Plasma 5-HIAA (nmol/l)	19.5 $\pm$ 7.2	22.5 $\pm$ 6.8	19.2 $\pm$ 6.1	19.5 $\pm$ 7.4	22.1 $\pm$ 7.8	0.074

<sup>1</sup> $P$  value by ANOVA test for repeated measurements for the main effect of time or  $t$  test for paired samples.

<sup>2</sup>Since ethinyl estradiol was administered 17 $\beta$ -estradiol was not assayed.

<sup>a,b</sup>Mean values within a row not sharing a common superscript letter were significantly different ( $P < 0.05$  by post-hoc Sidak test).

smoking status, BMI and creatinine levels as covariates. No sex difference was found for 5-HIAA ( $P = 0.981$ ; Fig. 2 and Table 1).

**Effects of cross-sex hormone administration** In M→F transsexuals, estrogen administration substantially decreased serum levels of testosterone, LH and FSH, whereas prolactin levels increased. The synthetic estrogen, ethinyl estradiol, could not be detected by the 17 $\beta$ -estradiol assay used (Table 1). There were no significant effects on plasma HVA and 5-HIAA (Fig. 2 and Table 1), although the change in plasma 5-HIAA was of borderline significance ( $P < 0.10$ ), though quantitatively small. The proportional increase in prolactin or decrease in testosterone, LH, FSH and total homocysteine were not related to proportional changes in plasma HVA or 5-HIAA.

In F→M transsexuals, testosterone administration increased serum levels of testosterone and creatinine levels, while serum levels of 17 $\beta$ -estradiol, LH and FSH were suppressed. Partly reflecting the anabolic effects, there were increases in BMI, lean body mass and plasma creatinine and homocysteine. Again, there were no significant effects on plasma HVA and 5-HIAA (Fig. 2), although there was a tendency for plasma 5-HIAA to increase of borderline significance ( $P = 0.074$ ; Table 1). The proportional changes in hormones, total homocysteine and creatinine were not related to the proportional changes in plasma HVA or 5-HIAA.

When baseline and 4-month values were used, there was no treatment effect when the two groups were combined in an ANOVA for repeated measurements, either for HVA ( $P = 0.79$ ) or for 5-HIAA ( $P = 0.21$ ). Thus, the effects of estrogen plus anti-androgen administration were not significantly different from the effects of testosterone administration. After 2 and 4 months of estrogen plus anti-androgen administration, smoking M→F transsexuals still had lower plasma HVA levels as compared with non-smoking M→F transsexuals ( $P < 0.01$ ). After 4 months of cross-sex hormone administration, there was still a lower mean level of plasma HVA in M→F transsexuals (genetic men) as compared with F→M transsexuals (genetic women) (15.2 nmol/l ( $P_{10}$  6.1;  $P_{90}$  37.5) vs 31.7 ( $P_{10}$  14.6;  $P_{90}$  72.7);  $P = 0.003$ ; Fig. 2 and Table 1).

## Discussion

Subjects in our study were (1) elderly men and postmenopausal women and (2) adult eugonadal genetic male and female transsexuals with sex-appropriate levels of sex steroids. In previous studies, women were found to have higher plasma levels of HVA than men (Bowers *et al.* 1983, Glazer *et al.* 1983, Bowers & Swigar 1987, Koreen *et al.* 1994, Davila *et al.* 1995, Szymanski *et al.* 1995, Sumiyoshi *et al.* 1997b), which could hypothetically be ascribed to their respective differences in sex steroid

milieus. However, this idea is not supported by our data. First, HVA levels in plasma were lower in elderly men than in postmenopausal women. This suggested that the sex difference is not affected by the decline in sex steroid levels as the physiological manifestations of aging, particularly following menopause. Remarkably enough, aging men have higher circulating levels of estradiol than postmenopausal women (Vermeulen *et al.* 2002). Secondly, administration of a high dose of oral ethinyl estradiol plus cyproterone acetate to genetic men and of testosterone to genetic women did not affect plasma HVA. Therefore, the male/female difference in plasma HVA cannot be explained by the male/female difference in sex steroid milieu, although an inverse correlation between HVA and testosterone levels was found in the genetic male group at baseline. These results are in line with the findings that testosterone administration in healthy young men did not affect HVA levels (Hannan *et al.* 1991), that women with polycystic ovary syndrome, which is associated with hyperandrogenism, have similar plasma levels of catecholamines and urinary levels of HVA as compared with female controls (Garcia-Rudaz *et al.* 1998), and that estrogen administration to postmenopausal women did not affect urinary HVA levels (Lobo *et al.* 1984).

What mechanism may explain the sex difference in HVA? Since sex steroids did not affect HVA, a putative explanation may be a sex differences in gene expression. MAO A and especially MAO B are responsible for the oxidative deamination of dopamine (Fig. 1), and are both coded for on the X chromosome (i.e. Xp11 region). There is evidence that MAO A and especially MAO B escape X-chromosome inactivation (Good *et al.* 2003). Since (genetic) women have two X chromosomes and MAO may be expressed from both X chromosomes, this would result in higher MAO activity and consequently higher HVA levels in women than in men. Alternatively, there are sex differences in the brain which develop and mature until early adulthood (Gogtay *et al.* 2004) and sex steroids may have produced lasting, so-called organizational, effects on dopamine-producing tissue during fetal, perinatal and pubertal periods. Previous studies in transsexuals also suggest that sexually dimorphic brain structures are resistant to exposure to cross-sex hormones and are not influenced by gonadal steroids during adulthood, but they have rather originated by direct action of the genetic factors (e.g. difference in gene dosage) or by the organizational effects of gonadal steroids during early development (Zhou *et al.* 1995, Kruijver *et al.* 2000).

We found that M→F transsexuals who were smokers had significantly lower plasma HVA as compared with non-smokers, which is in accordance with the finding that male smokers had lower cerebrospinal fluid levels of HVA (Geraciotti *et al.* 1999). Cigarette smoke may have substantially inhibited the central nervous system MOA activity, leading to increased dopaminergic activity (Fig. 1) (Brody *et al.* 2004) as well as lower plasma HVA. Alternatively,

men with low HVA levels may be more prone to become smokers (i.e. reverse causation). The increase in prolactin levels in M→F transsexuals upon estrogen administration is likely the reflection of a direct estrogenic enhancement or lower dopaminergic inhibitory effects on prolactin secretion. In previous studies in postmenopausal women, estrogen replacement therapy increased prolactin and cortisol responses to the serotonin agonist metachlorophenylpiperazine (Halbreich *et al.* 1995) and hormone replacement therapy increased prolactin secretion in response to the dopamine-blocking agent sulpiride (Paoletti *et al.* 2001). The findings were thought to reflect changes in serotonergic and dopaminergic activity upon estrogen administration (Halbreich *et al.* 1995, Paoletti *et al.* 2001). These estrogenic effects may be confined to the pituitary gland and the hypothalamic structures that control the pituitary production of prolactin, but the effects of sex hormones are likely to differ from one brain region to another. Dopamine degradation proceeds by MOA and COMT, which catalyze the transfer of a methyl group from methionine to dopamine, resulting in homocysteine and HVA (Fig. 1). Because the synthetic pathways are coupled, it is of note that the profound decrease and increase in plasma total homocysteine in M→F and F→M transsexuals respectively (Giltay *et al.* 2003) were not paralleled by changes in plasma HVA.

Methodologically, the use of plasma levels of HVA has its limitations. Plasma levels of HVA are dependent upon the balance between both delivery into the plasma and clearance from the plasma (half-life:  $\pm 40$  min) (Goldstein *et al.* 2003). Only 12–40% of HVA in plasma is of central nervous system origin (Swann *et al.* 1980, Maas *et al.* 1988, Lambert *et al.* 1991, Lambert *et al.* 1993, Amin *et al.* 1995, 1998); much more is derived from central and peripheral sympathetic nerves and the adrenal medulla. It is possible that sex steroids affected biosynthesis, transport, concentration, uptake, receptor potentiation and density, post-synaptic responsivity, blood–brain barrier, degradation, or clearance of dopamine and serotonin (Halbreich & Kahn 2001). Potential effects on dopamine and serotonin activity may have been obscured by compensatory (i.e. negative feedback) mechanisms, and by the indirect assessment of monoamine status in plasma samples. Finally, alterations in renal clearance may cause concomitant changes in the plasma HVA. However, the increase in plasma creatinine upon testosterone administration is not caused by a decrease in renal function, since creatinine clearance was unaffected by testosterone administration in another study of cross-sex hormone administration to transsexuals (Giltay *et al.* 1998). The increase in plasma creatinine is rather a reflection of the anabolic effect of testosterone in genetic females, as indicated by the increase in lean body mass.

The interpretation of our results is limited by the inclusion of a relatively small numbers of subjects. Furthermore, the intervention study had a relatively short follow-up of 4 months, had an open-label design and

lacked a true placebo group, due to the nature of the study population and the treatment indication. Moreover, different effects may have been found in hypogonadal men and women undergoing sex-appropriate hormone replacement. Yet, the dose of cross-sex hormone administration was high and the levels of HVA were consistently lower in men than in women as well as in M→F transsexuals than in F→M transsexuals, both before and after cross-sex hormone administration.

We found that fasting plasma levels of HVA, the major metabolites of dopamine, was not significantly changed by administration of high-dose cross-sex hormone administration in F→M and M→F transsexual subjects. Our results have suggested that the sex difference in plasma HVA – with higher HVA levels in females than in males – is related to mechanisms other than exposure to sex steroids in adulthood, and may rather be the result of a sex difference in genetic factors or of organizing effects of sex hormones during early development.

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