

Oestrogen-mediated hormonal imbalance precipitates erectile dysfunction

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Declining testosterone (T) in an aging male offsets the equilibrium between androgen and oestrogen (oestradiol, E₂) with a resultant increase in E₂-T ratio. Similar functional hormone imbalance is existent in clinical states of hypogonadism and is likely to arise from exposure of males to environmental oestrogens. The pathophysiological significance of this derangement on erectile function, hitherto unknown, was estimated in sexually mature male rats following acute and chronic treatment with oestrogen. A total of 60 male Sprague-Dawley rats (200–250 g) were divided into control and two treatment groups, administered 0.01 and 0.1 mg of oestradiol through oral gavage daily for 1 week ($n = 30$, acute study) and 12 weeks ($n = 30$, long-term study), respectively. Sexual activity in the presence of hormonally primed female rats and intracavernous pressure (ICP) response to electrical stimulation estimated treatment-induced changes, which were correlated with hormone levels and penile morphology at 12 weeks. Following two to five-fold elevation in serum E₂ levels (and simultaneous reduction in testosterone), there was a significant prolongation of mount, intromission, ejaculation latencies and some decrease in frequencies. The ICP response to nerve stimulation was also impaired in all the treated groups. Histologically, trichrome staining highlighted the cavernosal connective tissue hyperplasia in the long-term study groups. Results of this investigation indicate that oestradiol causes pathophysiological changes in erectile function. These observations provide an indirect evidence for the possible sexual health hazards in man upon inadvertent exposure to environmental oestrogens, ageing and derangement of E₂-T ratio. *International Journal of Impotence Research* (2003) 15, 38–43. doi:10.1038/sj.ijir.3900945

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Introduction

Sexual dysfunction with impotence and loss of libido is an important accompaniment of deranged testicular pathways and ageing in males.¹ Diagnostically, low serum testosterone (T) level correlates with impairment of penile sensitivity, nocturnal penile tumescence and spontaneous morning erections in man² and reduced cavernosal pressure response in the animal model.³ Besides clinical signs, the endocrine profile is further compounded by a functional excess of oestradiol (E₂) that offsets the delicate E₂-T balance.⁴ In such a situation, the widespread distribution of oestrogen receptors in

the male reproductive tract⁵ may mediate a state of biological oestrogenism by the 'female hormone'. While the need for oestrogen receptor-mediated action in the context of normal male fertility and sperm production is understood,⁶ to date there is no information on the possible modulating effect of oestrogen with regard to sexual function and dysfunction in men.

The testosterone decline with elderly hypogonadism in ageing has been delineated^{1,2} and linked to functional changes in the hypothalamo-hypophysial axis⁴ as well as decreases in Leydig cell number, enzymatic activity and sensitivity to gonadotropin stimulation.⁷ Furthermore, there is a significant elevation of E₂ level in these individuals.⁸ Similar pathophysiological imbalance between androgen and oestrogen is likely to be associated with other clinical states of adult-onset hypogonadism⁹ as well as hyperoestrogenism.¹⁰ Under such circumstances, since E₂ is a more potent gonadotropin suppressant than testosterone,⁴ a vicious cycle that leads to an absolute testosterone deficiency is likely to be precipitated by E₂-induced decrements in LH and FSH release. As testosterone

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level declines further and E_2 increases, the imbalanced relationship between these two hormones may differentially affect various parameters of sexual function. Moving from the mechanisms underlying the role of testosterone in erectile physiology, the sexual impairment secondary to E_2 or changes in its ratio with testosterone (E_2 -T) needs to be addressed. A preliminary study from our centre had indicated the detrimental effect of intravenous oestradiol on cavernous pressure response to nerve stimulation.¹¹ Subsequently, in this investigation, the pathophysiological significance of this endocrine derangement on erectile function was estimated in sexually mature male rats following an acute and chronic oral treatment with oestrogen.

Methods

A total of 60 male Sprague–Dawley rats (200–250 g) were divided into control and two treatment groups, administered 0.01 and 0.1 mg of oestradiol valerate (Progynova, Schering) suspension in distilled water through oral gavage daily, for 1 week ($n=30$, acute study) and 12 weeks ($n=30$, long-term study), respectively. Control rats consumed the same volume of distilled water, fed daily by gavage. The animals were housed under reversed 12 h light and dark cycle and controlled room temperature (21°C) with standard rat chow accessed *ad libitum*. Experimental procedures were conducted in absolute compliance with the international guiding principles for animal research. All the rats were assessed before and after treatment for changes in body weight.

Sexual behaviour

At the initiation of the experiment, all animals participating in the study were paired with proceptive female rats to record the baseline sexual activity for each group. Oestrous behaviour was induced in these ovariectomised female animals through subcutaneous administration of 30 µg of oestradiol benzoate (Sigma) and 1.5 mg of progesterone (Biological Italia), 48 and 4 h prior to the study, respectively.¹² Mating experiments were carried out in an isolated Perspex chamber (42 × 38 × 20 cm³) between 14.00 and 19.00 h, aided with dim red illumination. Following a 10 min adaptation period for the male rat, primed female rat was placed in the immediate vicinity and sexual behaviour recorded as individual events of mounts and intromissions leading to ejaculation.¹³ Counted parameters included mount, intromission and ejaculation latencies (ML, IL and EL), numbers of mounts and intromissions (MF and IF) and the postejaculatory

time interval for subsequent mounting (PEI). After recording the baseline function, these tests were repeated at the end of 1 week (acute study) and 6 and 12 weeks for animals of the long-term study group.

Cavernous pressure response

Following the treatment period, male rats were anaesthetised with pentobarbitone sodium (Sanofi, 45 mg/kg i.p. with 10 mg/kg i.v. supplement, when required). Right external jugular vein and left internal carotid artery were cannulated (E_{50} tubing), respectively, for drug administration and blood pressure (BP) monitoring. Trachea was cannulated for ventilation. Through a lower median incision, lateral prostate was exposed and the cavernous nerve carefully isolated and suspended by platinum wire electrode for stimulation.¹³ A 27-gauge needle filled with heparinised saline (250 IU/ml) was inserted into the penile crus (PE_{10} tubing) for recording intracavernous pressure (ICP) response to nerve stimulation. Recordings were obtained at the optimal voltage and frequency of 2 V and 20 hz over 30–45 s. Mean systemic blood and intracavernous pressure changes were measured with transducers (Ugo Basile) and recorded with MacLab analogue-digital converter (AD instruments).

Hormone levels

Blood samples for hormone estimation were collected from the carotid artery at the end of pressure recordings. Samples were stored at room temperature for 2 h and centrifuged at 3000 rpm and 15°C for 10 min. Separated serum was stored at -70°C until further analysis. Estimation of levels of sex hormone binding globulin, free oestradiol and free testosterone could not be carried out in this component of our study. However, the levels of total testosterone and total oestradiol were measured by the scintillation proximity radioimmunoassay to identify the changing trends in hormonal status following treatment.

Histology

At the end of *in vivo* studies, penile tissue from rats of long-term subgroups was prefixed in Bouin's solution, embedded in paraffin and processed prior to Masson's trichrome staining for visualisation of structural details under light microscopy.¹⁴

Statistical methods

Data were expressed as mean \pm s.e.m. and analysed by one-way ANOVA and Tukey's test for multiple comparisons. Evaluation for significant difference from the control results was set at $P < 0.05$.

Results

Within 1 week of oral oestradiol intake, the sexual behaviour and body weight fluctuations remained statistically insignificant from the control values, while the body weight, however, reduced significantly in the higher E₂ (0.1 mg) treatment group at 12 weeks (data not shown). Consequent to two to five-fold elevation in serum E₂ levels and simultaneous decrements in testosterone (Table 1) indicative of possible trends in free hormone levels, the male rats of higher oestradiol treatment group showed an impairment of certain parameters of mating behaviour. Statistically significant changes included prolongation of intromission and postejaculatory mounting latencies (Figures 1b and 2b). Extended ejaculatory latency (0.1 mg, Figure 2a) and reduced intromission frequency (0.01 mg, Figure 3b) were disregarded due to similar differences from the other two groups at the initiation of the study. Histologically, a loose connective tissue replaced smooth muscle fibres in the cavernosum of oestrogen-treated rats at 12 weeks (light micrographs, Figures 4(a-c)). During *in vivo* cavernous nerve stimulation studies, an optimal ICP elevation of 43.93 ± 2.56 mmHg was obtained in control rats at standard experimental criteria of 20 Hz, 2 V and 30–45 s impulse duration. However, in the E₂-treated groups, the functional response to stimulation was significantly impaired at the higher dose and the mean ICP increases at peak stimulation were 41.27 ± 1.78 mmHg (0.01 mg) and 36.33 ± 0.39 mmHg (0.1 mg), respectively. These results were further substantiated by significantly reduced ICP response to the E₂ administration of 1 week, viz., 39.47 ± 0.49 mmHg (0.01 mg) and

Table 1 Serum levels (ng/ml) of total E₂ and T in the different experimental groups. E₂-T ratio gives the hormonal imbalance arising from oral treatment with E₂

	Oestradiol	Testosterone	E ₂ -T Ratio
Control (1 week)	0.024 \pm 0.003	3.467 \pm 0.482	0.7
E ₂ : 0.01 mg, 1 week	0.077 \pm 0.010	2.737 \pm 0.932	2.8
E ₂ : 0.1 mg, 1 week	0.113 \pm 0.020*	1.011 \pm 0.276*	11.2
Control (12 weeks)	0.030 \pm 0.003	3.100 \pm 0.453	0.9
E ₂ : 0.01 mg, 12 weeks	0.049 \pm 0.004	0.980 \pm 0.157*	5.0
E ₂ : 0.1 mg, 12 weeks	0.109 \pm 0.016*	0.496 \pm 0.118*	21.9

*Statistical significance by one-way ANOVA is $P < 0.05$.

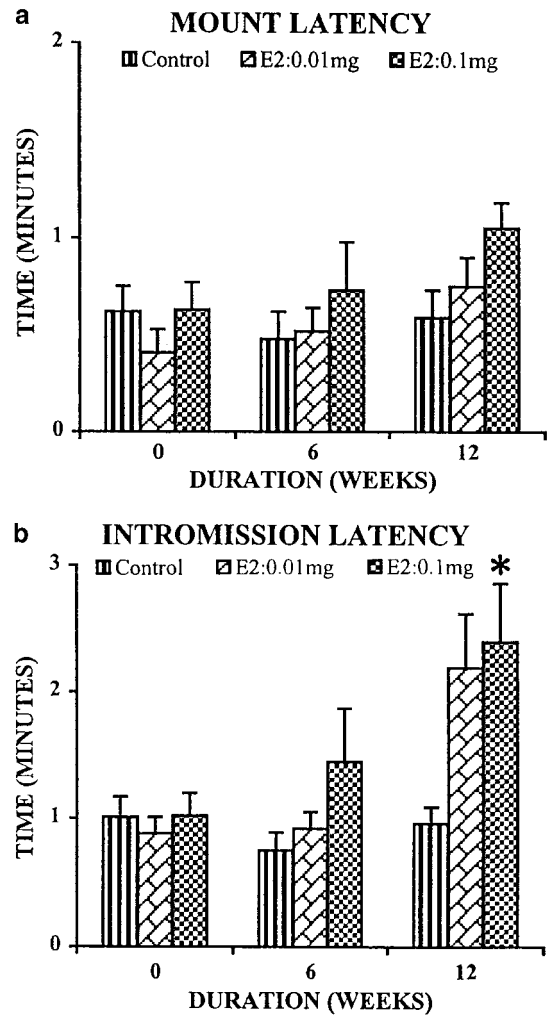


Figure 1 First ML (a) and IL (b) in the different experimental groups of rats ($n = 10$). Statistical significance by one-way ANOVA is * $P < 0.05$.

34.44 ± 1.50 mmHg (0.1 mg), as compared to a control value of 45.28 ± 1.20 mmHg (Table 2).

Discussion

Results of this investigation provide the first preliminary evidence for E₂-induced alterations in erectile function through studies on sexual behaviour and cavernous nerve stimulation criteria. Dose-dependent and centrally mediated weight reduction follows oestradiol intake,¹⁵ while its impact on testosterone is more complex, mediating through negative feedback and low LH levels.^{4,15} While an ejaculatory dysfunction was precipitated in mice by oestrogen alpha-receptor inactivity,¹⁶ high oestradiol intake for 12 weeks compromised arousal, desire (mounting) as well as sexual performance (intromission) in one of our treatment groups

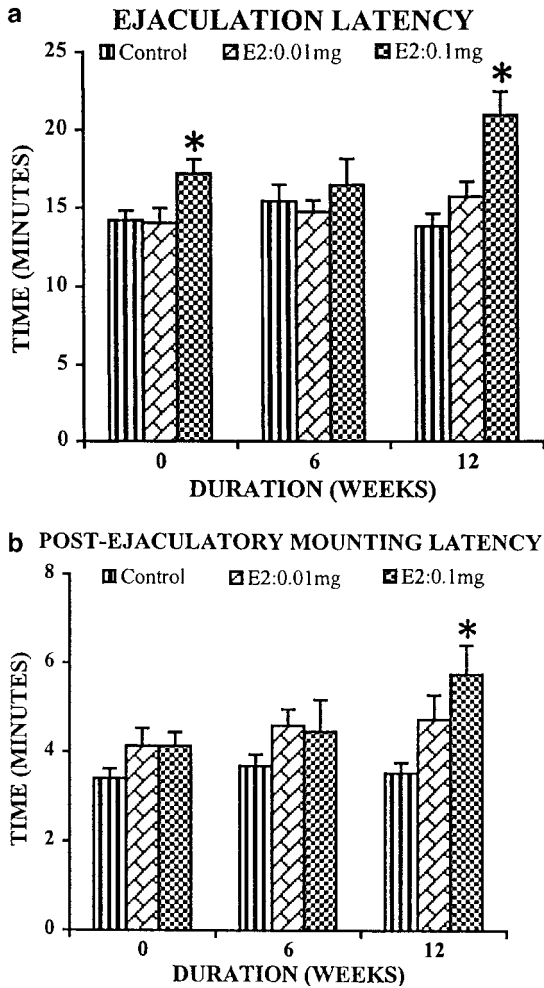


Figure 2 EL (a) and subsequent ML (b) in the different experimental groups of rats ($n=10$). Statistical significance by one-way ANOVA is $*P<0.05$.

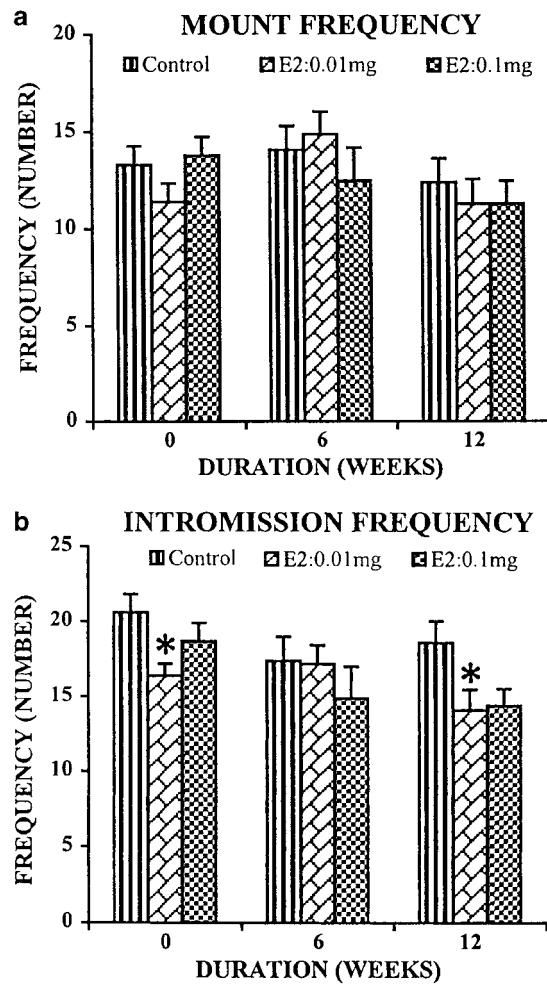


Figure 3 Total number of mounts (a) and successful intromissions (b) in the different experimental groups of rats ($n=10$). Statistical significance by one-way ANOVA is $*P<0.05$.

(0.1 mg). Conforming to structural changes in the cavernosum of trans-sexuals exposed to oestrogen,¹⁷ the penile morphology demonstrated a reduction in smooth muscle and relative increase in connective tissue distribution. Intracavernous pressure response to nerve stimulation is identified to be an index for the quantitative assessment of penile erection in animal models.¹⁸ Thus, the impaired pressure response to preliminary intravenous¹¹ as well as oral E₂ administration in the present investigation is presumably due to a detrimental effect of oestrogen on neuronal nitric oxide (NO)-mediated response of penile tumescence and rigidity; the mechanism of this action needs further investigation and elucidation.

Our results indicate that the changes are brought about by a disruption in hormonal milieu coinciding with elevated E₂ level, decrease in circulating testosterone and the resultant E₂-T imbalance. This derangement may also initiate a simple, nevertheless direct, functional antagonism by oestrogen of

testosterone-mediated events of erectile response. The secondary hypogonadal state (relative testosterone deficiency) identified in our male rats is possibly precipitated and aggravated by oestrogenic gonadotropin-suppressive effect (feedback inhibition of LH and FSH release), mentioned earlier.^{4,15} This may further compound the pathophysiological changes of endocrine imbalance. Although clinically¹⁹ and experimentally,²⁰ testosterone supplementation improves senile changes in sexual function to a moderate extent, a true causal approach may further envisage concurrent management of rising oestrogen level in the affected individuals. For instance, oestradiol reduced sperm motility in rats,²¹ and antiestrogen tamoxifen had promoted spermatogenesis in an anecdotal case report of incomplete androgen insensitivity accompanied by elevated E₂ levels.²²

Another related sexual health concern of the community at large is the oestrogenic influence of compounds of environmental origin including

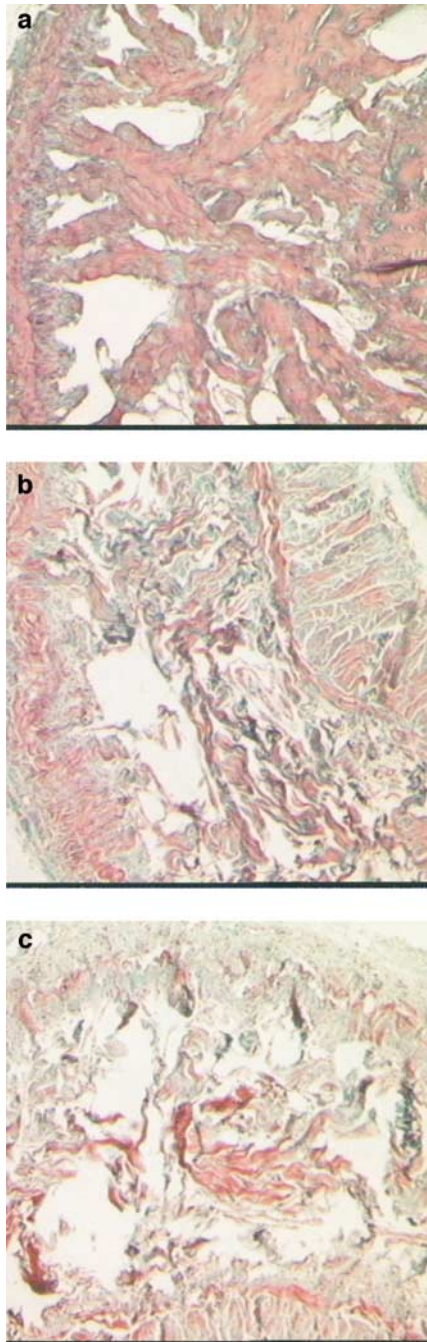


Figure 4 (a) Light micrograph 1: trichrome-stained light micrograph of control rat cavernosum. Scattered sinusoids, smooth muscle fibres and connective tissue show normal architecture (magnification: $\times 50$). (b) Light micrograph 2: trichrome-stained light micrograph of cavernosum from E_2 -(0.01 mg) treated rat at 12 weeks. Some degree of connective tissue proliferation is seen (magnification: $\times 50$). (c) Light micrograph 3: trichrome-stained light micrograph of cavernosum from E_2 -(0.1 mg) treated rat at 12 weeks. There is extensive loose connective tissue proliferation amidst the distribution of sinusoidal spaces and scanty smooth musculature (magnification: $\times 50$).

phytoestrogens.²³ Based on our results, we believe that a chronic exposure of male population to these

Table 2 Cavernous and mean systemic BP recordings (mmHg) at electrical stimulation in the different experimental groups

	ICP	BP
Control (1 week)	45.28 \pm 1.20	88.54 \pm 4.76
E_2 : 0.01 mg, 1 week	39.47 \pm 0.49*	110.71 \pm 4.93
E_2 : 0.1 mg, 1 week	34.44 \pm 1.50*	103.77 \pm 12.26
Control (12 weeks)	43.93 \pm 2.56	83.32 \pm 21.98
E_2 : 0.01 mg, 12 weeks	41.27 \pm 1.78	94.57 \pm 10.91
E_2 : 0.1 mg, 12 weeks	36.33 \pm 0.39*	114.53 \pm 16.92

*Statistical significance by one-way ANOVA is $*P < 0.05$.

chemical substances may adversely affect their libido and erectile function due to endogenous hormonal modulation, similar to the changes in this experimental scenario. Along these lines, the role of phytoestrogens on libido and erectile physiology is being evaluated currently.

Conclusion

This study provides the first direct measurement of declining erectile function on exposure to oestradiol and the resultant E_2 -T imbalance in a pathophysiological situation. The results will have pertinent clinical implication in the diagnosis and management of impotence related to endocrine derangements. Furthermore, the findings predict the likelihood of erectile dysfunction from inadvertent exposure to environmental oestrogens.

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