Post-translational regulation of endothelial nitric oxide synthase by testosterone in the mouse penis

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Introduction
Nitric oxide (NO) is the main mediator of penile erection.\textsuperscript{1} In endothelial cells, NO synthesis is catalysed by endothelial NO synthase (eNOS). eNOS phosphorylated at Ser-1177, a positive regulatory site, constitutively increases NO production, which, via vasorelaxation of the cavernosal smooth muscle cells, maintains penile erection.\textsuperscript{2} eNOS phosphorylation at Ser-1177 is stimulated by several stimuli including shear stress, oestradiol, vascular endothelial growth factor and, potentially, testosterone.\textsuperscript{3} Testosterone is essential for the normal structure and function of penile cavernous tissue.\textsuperscript{4} In hypogonadism, low levels of testosterone are associated with erectile dysfunction and treatment with testosterone improves erectile function.\textsuperscript{5} Recent studies have shown that testosterone beneficially regulates the vasculature.\textsuperscript{5} However, the precise vasoprotective mechanisms of testosterone in the penis are unknown. We hypothesise that testosterone acting through the Akt pathway positively regulates eNOS phosphorylation at Ser-1177, and the subsequent increase in NO production promotes penile vascular homeostasis and penile erection (Figure 1).

Methods
Eight-week-old wild-type mice were used (n=15). Twelve mice were castrated and split into four groups of three. Pellets were implanted subcutaneously, releasing 1.2\(\mu\)g, 12\(\mu\)g or 70\(\mu\)g of testosterone or...
vehicle per day in each group. Three non-castrated mice were used as controls. After three days, total serum testosterone levels were measured by radioimmunoassay, and protein levels of phosphorylated-eNOS (p-eNOS), eNOS, phosphorylated-Akt (p-Akt) and phosphorylated vasodilator-stimulated phosphoprotein (p-VASP) were investigated by western blotting in the penises excised at baseline. All results were expressed relative to non-castrated mice.

FIGURE 3: Western blot demonstrating p-eNOS (Ser-1177) and eNOS protein levels in the penises of non-castrated control mice, and castrated mice treated with vehicle, 1.2µg, 12µg or 70µg of testosterone (T)/day for three days. Lower panels represent quantitative analysis of P-eNOS (Ser-1177)/eNOS in penises in the same treatment groups. Each bar represents the mean ± SEM of three mice.

FIGURE 4: Western blot demonstrating eNOS and β-actin protein levels in the penises of non-castrated control mice, and castrated mice treated with vehicle, 1.2µg, 12µg or 70µg of testosterone (T)/day for three days. Lower panels represent quantitative analysis of eNOS/β-actin in penises in the same treatment groups. Each bar represents the mean ± SEM of three mice.

FIGURE 5: Western blot demonstrating p-Akt and Akt protein levels in the penises of non-castrated control mice, and castrated mice treated with vehicle, 1.2µg, 12µg or 70µg of testosterone (T)/day for three days. Lower panels represent quantitative analysis of p-Akt/Akt in penises in the same treatment groups. Each bar represents the mean ± SEM of three mice.

FIGURE 6: Western blot demonstrating p-VASP and VASP protein levels in the penises of non-castrated control mice, and castrated mice treated with vehicle, 1.2µg, 12µg or 70µg of testosterone (T)/day for three days. Lower panels represent quantitative analysis of p-VASP/VASP in penises in the same treatment groups. Each bar represents the mean ± SEM of three mice.
Results
Treatment with 1.2 μg of testosterone p/day resulted in physiological serum testosterone levels (1.6 pm/ml), whereas 12 μg of testosterone p/day and 70 μg of testosterone p/day resulted in supraphysiological serum testosterone levels (7.9 pm/ml and 13.5 pg/ml, respectively) (Figure 2). Consistent with our hypothesis, testosterone deficiency showed a trend towards reduced eNOS phosphorylation at Ser-1177 (p=0.1124), while administration of low and medium doses of testosterone increased eNOS phosphorylation above castrated levels (Figure 3). Testosterone deficiency showed a trend towards increased eNOS protein expression levels (p=0.3807), while different testosterone doses showed no effect on eNOS protein expression compared to non-castrated mice (Figure 4). P-Akt levels exhibited changes similar to p-eNOS (Ser-1177) in response to castration and testosterone replacement, suggesting that testosterone-induced eNOS phosphorylation at Ser-1177 occurs through the p-Akt pathway (Figure 5). Testosterone did not affect protein expression of p-VASP, a marker of NO signalling (Figure 6).

Conclusion
In the mouse penis, testosterone may induce eNOS phosphorylation at Ser-1177 via the p-Akt signalling pathway. This may promote penile vascular homeostasis and positively influence penile erection.

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References