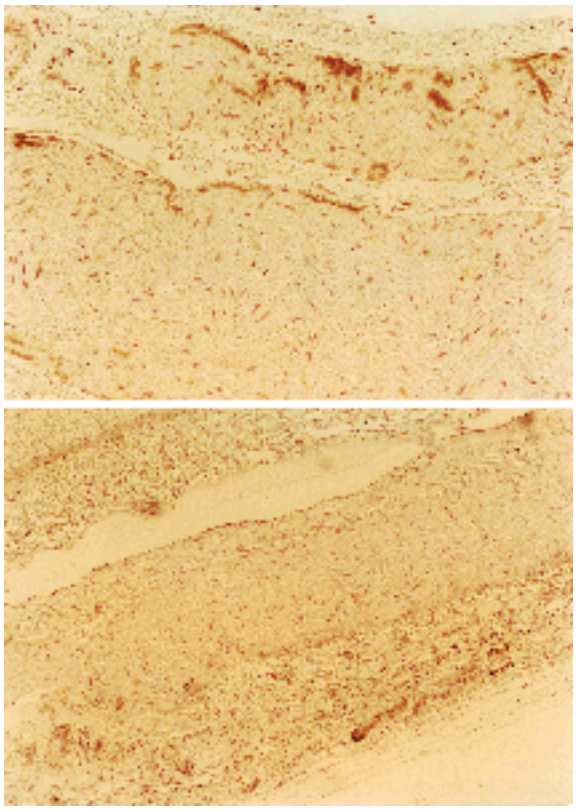
polyclonal antibody revealed the presence of nNOS in the nerves. The staining in 14-week-old rats was strong (Fig. 2A), and the staining in 15-month-old rats with testosterone replacement was almost the same in level as in 14-week-old rats (Figs. 2D and E). In 15-month-old rats without testosterone replacement, nNOS was weakly positive (Fig. 2C).

Discussion

The results of this study using young adult and middle-aged rats showed that there was an age-related decrease in serum testosterone concentration, NO level and NOS expression in the corpus cavernosum, and moreover, that in middle-aged rats with 2 months of testosterone replacement which was continued until the rats reached the age of 15 months, those levels were restored to the level in young adult rats. Several reports show that a low testosterone level decreases penile erection and treatment with testosterone restores it (Mills et al., 1992; Heaton and Varrin, 1994; Meisel and Sachs, 1994; Garban et al., 1995). One important question is what is the role of testosterone by itself in the maintenance of penile erection. The author has significantly extended these previous findings by demonstrating that the most likely mechanism of penile dysfunction is an aging-induced reduction in the level of penile NO and NOS, a reduction which is prevented by testosterone.

Recently, several physiological studies (Holmquist et al., 1991; Burnett et al., 1992) of electrically induced erections as well as pharmacological studies (Ignarro et al., 1990; Kim et at, 1991; Rajifer et al., 1992) have indicated that NO plays a major role in the mediation of penile erection. Penile erection results from relaxation of the corporal smooth muscle and penile cavernosal arteries. NO mediates this process as supported by evidence showing that NO has a direct vasodilatory effect on cavernosal tissue and that relaxation of penile smooth muscle is prevented by NOS inhibitors (Krane et al., 1989; Seftel et al., 1994; Burnett, 1997). The production of NO is mediated by a family of NOS that all represent distinct gene products (Forstermann et al., 1995). There are 3 iso-



A: G1, young adult rat *(14-week-old)*
B: G2, middle-aged rat *(13-month-old)*

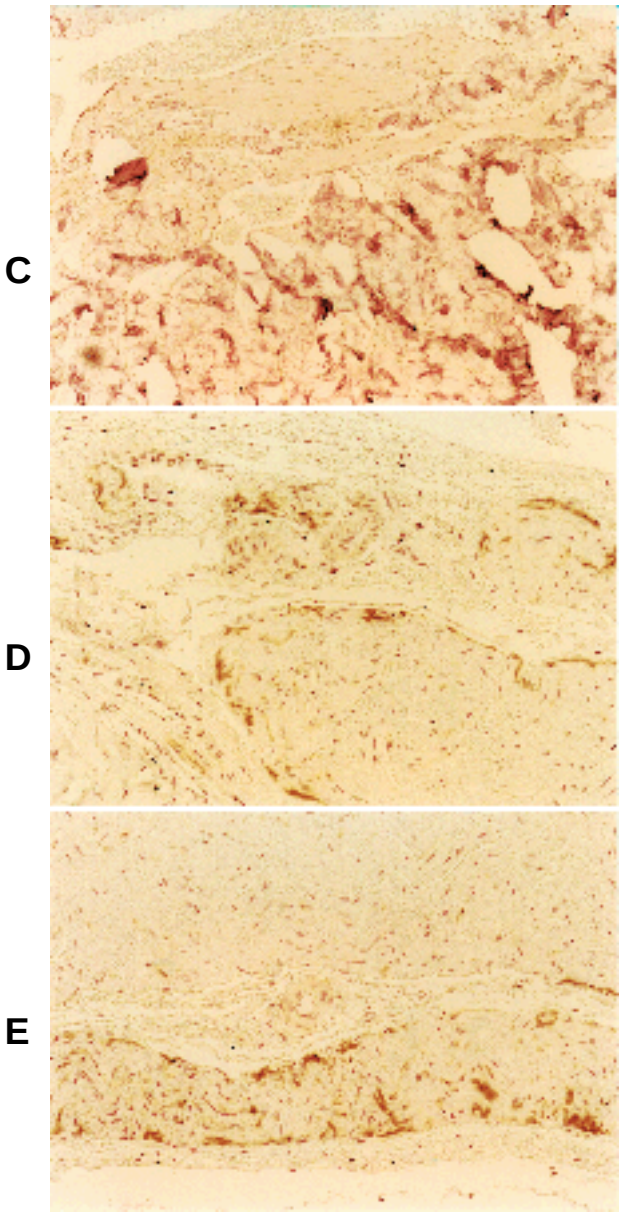
Fig. 2. Sections of rat corpus cavernosum tissue immunostained by ant-neuronal nitric oxide synthase (nNOS) antibodies (original magnification × 200).
A, D and E: Nerve fibers are stained at the same level in G1, G4 and G5.
C: Staining is weaker than that of the young adult group.

[Figs. 2A and B on p. 50 and Figs. 2C–E on p. 51]

forms, 2 constitutive and 1 inducible, which differ in their dependence on intracellular calcium.

Of the constitutive isoforms, eNOS was first identified in bovine aortic endothelial cells and appears to play an important role in the transduction of signals from the bloodstream to the underlying smooth muscle which causes vasorelaxation (Lamas et al., 1992; Sessa et al., 1992). The expression of eNOS protein and mRNA has been examined in several disease processes. Increased expressions of eNOS mRNA and protein has been found in atherosclerotic vessels and in response to shear stress as well as estrogen (Kanazawa et al., 1992; Nishida et al., 1992; Weiner et al., 1994). Increased eNOS activity has been found in causes of renal failure, whereas decreased activity has been found in pulmonary hypertension (Weiner et al., 1994; Conger et al., 1995). These suggest that the eNOS expression is varied and that its expression is a function of the offending disease process. eNOS has been thought to be a putative key enzyme of penile erection in that acetylcholine stimulates the release of endothelium-derived relaxation factors from intact rabbit cavernosal smooth muscle cells (Seftel et al., 1994) and also induces the relaxation of penile smooth muscle, a condition which is reversed by NOS inhibitors (Kim et al., 1991). Furthermore, impaired endothelium-dependent corporal smooth muscle relaxation has been found in diabetic men with erectile dysfunction (Saenz et al., 1989). The recent immunohistochemical localization of eNOS in the endothelial layers of the dorsal penile arteries, veins and corporal sinusoids places the enzyme in a physiologically critical location for mediating penile erection (Burnett et al., 1996).

nNOS has been localized in the rat penile autonomic nerves, the adventitia of rat penile arterioles, and the autonomic innervation of the human penis (Burnett et al., 1993). It has also been reported that there are



C: G3, middle-aged rat
D: G4, low-dose testosterone replaced middle-aged rat
E: G5, high-dose testosterone replaced middle-aged rat
(G3–G5: 15-month-old)

Figs. 2C–E. *Continued from the previous page.*

numerous NOS-containing nerve fibers and terminals innervating corpus cavernosum smooth muscles and vessels in the spaces of the corpus cavernosum.

The presumed role of nNOS in penile erection has been studied in rats by using electrical stimulation of the pelvic nerves, which caused penile erection that was prevented by the NOS inhibitor L-nitroarginine methyl ester (Burnett et al., 1992, 1996; Bush et al., 1992). The NOS activity (presumed to be nNOS) has been indirectly measured in penile tissue homogenates by using a [^{3}H]-L-arginine to [^{3}H]-citrulline conversion assay, which, along with Western blotting analysis, showed a decrease in penile NOS activity and penile nNOS protein content in diabetic rats with erectile dysfunction (Vernet et al., 1995). A recent demonstration that nNOS-deficient transgenic mice maintain a normal penile erection throughout eNOS-dependent production of NO suggests that eNOS may also have an important primary and compensatory role in mediating penile erection (Burnett et al., 1996).

Previous studies have shown a decreased penile NOS activity in models of erectile dysfunction, such as hypogonadism, androgen receptor blockade and diabetes (Burnett et al., 1992). Our model of middle-aged rats had a low level of the penile NOS expression. Lugg et al. (1995) reported that in rats, aging induced a considerable reduction in the erectile response to electric field stimulation which was accompanied by a decrease in NOS activity in very old rats. Several reports have shown that a low testosterone level decreases penile erection and testosterone restores it (Mills et al., 1992; Heaton and Varrin 1994; Meisel and Sachs, 1994; Garban et al., 1995).

The results of this study showed that total and free testosterone levels tended to decrease with aging, simultaneously with the penile NO concentration and NOS expression. Testosterone replacement restored these levels. As mentioned above, testosterone may play a major role in the mediation of penile erection by affecting the pathway relaxing the corpus cavernosum smooth muscle in a manner that is dependent on the NOS activity. It is believed that testosterone replacement might be an effective method of treating erectile dysfunction that occurs with aging.

In summary, the serum testosterone concentration, penile NO concentration and NOS expression decreased with aging and were restored by testosterone replacement. According to the literature, NO plays a major role in the mediation of penile erection, and the present results suggest that testosterone replacement might be an effective method of treating erectile dysfunction that occurs with aging.

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