Aging Impairs Nitric Oxide-Mediated Relaxant Responses of Rabbit Corporal Smooth Muscle

Tijen Utkan,* Şahin Yıldırım,† Yusuf Sarioglu,† Furuzan Yildiz,* and Kemal Yıldırım†

*Department of Pharmacology, Faculty of Medicine, Kocaeli University, 41900 Derince Kocaeli, Turkey; and †Department of Pharmacology, Faculty of Medicine, Cumhuriyet University, 58140 Sivas, Turkey

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Aging has been reported to cause impotence, the mechanism of which is unknown. Therefore, we investigated the effect of aging on electrical stimulation-induced neurogenic, carbachol-induced, endothelium-dependent, and sodium nitroprusside-induced cGMP-dependent relaxant responses of rabbit corporal smooth muscle. Male New Zealand white rabbits were divided into young (4 months), intermediate (8 months), and old (24 months) groups. Electrical stimulation-, carbachol-, sodium nitroprusside-, and papaverine-induced relaxant response in isolated corporal smooth muscle strips were determined using in vitro muscle technique. Although there was no significant difference in the relaxant response of corporal strips to papaverine among the groups, relaxant responses to carbachol and sodium nitroprusside were significantly lower in corporal strips of old group than both young and intermediate groups. However, in the old and intermediate groups, electrical field stimulation-induced neurogenic relaxation was significantly reduced compared with the young group. KCl-induced (124 mM) contractile responses were the same in all groups. Our data indicate that the presence of age-dependent differences in the NO/cGMP-mediated relaxant responses of corporal tissue in the male rabbits. This may contribute to the development of impotence.© 2002 Elsevier Science (USA)

Key Words: penis; muscle; smooth; aging; rabbits; impotence.

Materials and Methods

Animals. Adult male New Zealand White rabbits (3–3.5 kg) were divided into three groups according to age: young, 4 months (n = 9); intermediate, 8 months (n = 9); and old, 24 months (n = 9). All animals anesthetized with ketamine (25 mg/kg, i.p) and xylazine (5 mg/kg, i.p.) and exsanguinated, as previously described (14). Briefly, the penis was dissected free and removed at the level of the crural attachments to the puboischial bones. The specimen was immediately placed in Krebs-bicarbonate solution composed of (mM):NaCl 118, KCl 4.7, CaCl₂ 2.5, NaHCO₃ 25, MgSO₄ 1.2, KH₂PO₄ 1.2, glucose 11. The tunica albu-
ginga was cleared of overlying tissue and opened. The proximal half of the corporal body was dissected free from the tunica and harvested en block.

Strip preparation and organ bath studies. Each corporal body was cut transversely to obtain two longitudinal strips. Each strip was mounted in a 20-ml organ bath containing Krebs-bicarbonate solution equilibrated with a gas mixture of 95% O2 and 5% CO2 and maintained at 37°C. Recordings of isometric strip tension were made using an FT03 transducer and recorded on a Grass model 79E polygraph (Grass Instruments, Quincy, MA). After mounting, each strip was allowed to equilibrate with a basal tension of 2 g for 1 h. Two grams of basal tension was chosen for the demonstration of reproducible relaxation response to field stimulation.

Cumulative concentration-responses. Rabbit corpus cavernosum strips in organ chambers were contracted with 10^-5 M phenylephrine and added to carbachol (10^-8–10^-4 M), sodium nitroprusside (10^-8–10^-4 M) and papaverine (10^-6–10^-4 M).

Transmural electrical stimulation (EFS). Transmural electrical stimulation was provided by a stimulator (Grass S 88) and applied via two platinum wire electrodes set vertically within the opposite organ bath sides of the suspended tissue. Prior to electrical stimulation, tissue was treated with guanethidine (5 µM) (adrenergic nerve blocker) and atropine (1 µM) (muscarinic receptor blocker) for 30 min. Strips were precontracted with phenylephrine at 10^-5 M, electric stimulation was then performed. Square-wave pulses of 10 V, 0.5 ms duration in 10-s trains with varying frequency (2–32 Hz) were applied at 5-min intervals. The strips were allowed to return to baseline precontractile tension between the tests at each frequency. Two or three agonists were tested on each preparation.

Analysis of data. The results are expressed as the mean ± SE of different experiments. Relaxant effects of agonists were expressed as percentage of the precontraction to phenylephrine. To evaluate the effects of agonists, pD2 (i.e., the negative logarithm of the concentration for the half-maximal response; EC50) and maximum response (E_max) values were calculated. The concentration-response data obtained in each individual experiment were plotted as the response/concentration (y) against the response (x). This produced a straight line relationship for each experiment, as predicted from the Scatchard equation for drug-receptor interactions.

Statistical comparisons between groups were performed using ANOVA followed t test. Probabilities of less than 5% (P < 0.05) were considered significant.

Drugs. The following drugs were all obtained Sigma Chemical: Carbachol chloride, phenylephrine hydrochloride, sodium nitroprusside, atropine sulfate, guanethidine sulfate, papaverine hydrochloride. All drugs dissolved in distilled water and freshly prepared on the day of the experiment.

Ethical approval was granted by the Kocaeli University Ethics Committee (Kocaeli, Turkey) (REC 60/3 Project No. 51).

**RESULTS**

The contractions elicited by KCl (124 mM) were similar in three groups (Table 1). Carbachol, sodium nitroprusside, and papaverine produced concentration-dependent relaxation in submaximally (70–75% of maximal contraction) precontracted (10^-5 M phenylephrine) corpus cavernosum strips obtained from young, intermediate, and old group. Endothelium-dependent relaxation to carbachol was significantly decreased in the old group compared with the intermediate and young group (P < 0.05). The concentration-response curve for carbachol was shifted to the right with significantly lower E_max and pD2 values (P < 0.05) (Fig. 1; Tables 1 and 2). Relaxation of cavernosal tissue to the NO donor sodium nitroprusside in the old group was decreased with significantly lower E_max and pD2 values compared with intermediate and young rabbits.

**TABLE I**

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Young</th>
<th>Intermediate</th>
<th>Old</th>
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<tbody>
<tr>
<td>Carbachol</td>
<td>76.4 ± 5.2</td>
<td>75.8 ± 4.3</td>
<td>36.4 ± 6.3*</td>
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<tr>
<td>Sodium nitroprusside</td>
<td>97.6 ± 2.3</td>
<td>96.7 ± 3.1</td>
<td>74.1 ± 4.1*</td>
</tr>
<tr>
<td>EFS</td>
<td>96.6 ± 7.4</td>
<td>70.1 ± 3.7**</td>
<td>16.3 ± 4.3*</td>
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<tr>
<td>Papaverine</td>
<td>98.6 ± 1.6</td>
<td>97.3 ± 3.2</td>
<td>96 ± 4.7</td>
</tr>
<tr>
<td>KCl</td>
<td>1300 ± 255.8</td>
<td>1295.3 ± 455</td>
<td>1150 ± 384</td>
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</tbody>
</table>

Note. Values are arithmetic means ± SE, n = the number of preparations used. *P < 0.05, statistically different from the response from young and intermediate-aged rabbits. **P < 0.05, statistically different from the response of strips from young and old rabbits.
Papaverine-induced relaxant responses was similar in all groups and there were no significant changes in the $E_{\text{max}}$ and $pD_2$ values. (Tables 1 and 2). Relaxation of cavernosal strips to carbachol, sodium nitroprusside and papaverine in the intermediate group was similar to the young group (Figs. 1 and 2; Tables 1 and 2). In precontracted strips, electrical field stimulation (2–32 Hz) evoked frequency-dependent relaxation. EFS-induced relaxation in cavernosal tissue from the old and intermediate group was significantly reduced compared with young group ($P < 0.05$) (Fig. 3; Table 1). When tissue contraction was produced with phenylephrine for the study of relaxation to various stimuli, the tension induced was similar in the three groups (data not shown).

### TABLE II

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<tbody>
<tr>
<td>Carbachol</td>
<td>6.62 ± 0.05</td>
<td>6.74 ± 0.07</td>
<td>5.62 ± 0.04*</td>
<td>5.45 ± 0.07</td>
<td>5.62 ± 0.08</td>
<td>4.64 ± 0.06*</td>
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<tr>
<td>Sodium nitroprusside</td>
<td>5.45 ± 0.07</td>
<td>5.62 ± 0.08</td>
<td>4.64 ± 0.06*</td>
<td>4.52 ± 0.06</td>
<td>4.68 ± 0.07</td>
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<tr>
<td>Papaverine</td>
<td>4.52 ± 0.06</td>
<td>4.68 ± 0.07</td>
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Note. Values are arithmetic means ± SE, n = the number of preparations used. *$P < 0.05$, statistically different from the response from young and intermediate-aged rabbits.

**DISCUSSION**

This study demonstrates inhibition of neurogenic, endothelium-dependent and a cyclic guanosine monophosphate (cGMP)-dependent direct smooth muscle relaxations of corporal smooth muscle in aging, thus confirming previous reports (12, 13). Endothelium-dependent relaxation to carbachol and electrical field stimulation of isolated rabbit corpus cavernosum strips precontracted with phenylephrine $10^{-5}$ M. Each point expressed as a percentage of the contraction induced by phenylephrine and is given as the mean ± SE of the mean. Numbers in parentheses indicate the number of preparations used. *$P < 0.05$, statistically different from the response from young and intermediate-aged rabbits.
stimulation-induced neurogenic relaxation of cavernosal tissue are mediated largely by the NO/cGMP pathway (7–9). The endothelium and autonomic nerves are independent sources of NO, mediating corpus cavernosum smooth muscle relaxation. Upon sexual stimulation, NO released by cavernosal nonadrenergic noncholinergic nerves and the endothelium activates guanylate cyclase in cavernosal smooth muscle, resulting in an increase in intracellular cyclic guanosine monophosphate (cGMP). The accumulation of cGMP leads to smooth muscle relaxation (8). In our study, neurogenic relaxation was impaired in both the old and intermediate group. The most likely explanation was impaired production of NO in the normal aging process and these changes appear to begin by middle age. In the old group, the reduced responses both endothelium-dependent and nerve-mediated relaxation suggest a possible common pathophysiologic mechanism with alteration in the nitric oxide/cGMP pathway. In old animals, tissue relaxation by the NO donor sodium nitroprusside was decreased compared to that of young and intermediate tissues. This confirmed that cavernosal tissue from old group impaired the ability to relax via the NO/cGMP pathway following exposure to NO donor agents. It is also possible that aging may impair relaxation of corporal smooth muscle. However, this possibility is unlikely since corporal strips relaxed well to papaverine. In addition, there were no differences in the KCl-induced contractile responses among the groups. Thus the contractile mechanisms were intact in the cavernosal smooth muscle. It is also important to note that at the concentrations of phenylephrine used, developed tension was similar for corporal strips from young, intermediate, and old animals, thus ensuring that any difference in relaxation among the groups was not due to differences in the degree of precontraction. Therefore, it is speculated that aging impairs the synthesis or availability of NO in corpus cavernosum tissue or the ability of erectile tissue to relax to NO. This is consistent with the results of previous reports on old rats. Authors reported that in experimental elderly animals the erectile response to nerve stimulation is diminished (12, 15) and that the number of NOS containing nerve fibers was significantly less in the old rats than in the young and intermediate-aged (16). Investigators believe that this is responsible for the delay in the onset of the intracavernous pressure rise after electrostimulation (16). In addition, it is suggested that aging causes an erectile failure due to factors initially independent from an impairment of penile NO synthesis but which is compounded in the very old rats by decrease of penile NOS activity (15).

An increasingly popular theory is that formation advanced glycosylation-end products have an important pathophysiologic role in the erectile dysfunction in aging and diabetes (17, 18). Alteration of endothelium-mediated relaxation may be the result of quenching and inactivation of NO by advanced glycosylation-end products (19). Nonenzymatic glycosylation of proteins, often referred to as the Maillard reaction, has been proposed to play a role in age- and diabetes-related process by forming protein and DNA links may contribute to erectile dysfunction by scavenging NO, which is needed for erection (20).

As is well known, both free estradiol and the binding protein TeBG increase with aging, altering the free estradiol:testosterone ratio. This may not only inhibit testosterone production but also affect LH and FSH secretion (5, 6). In rats, orchidectomy results in reduced nitric oxide synthase activity (21) and estradiol decreases NOS II synthesis (22). Therefore it is speculated that aging-induced impaired neurogenic and endothelium-dependent relaxation may be due to the increase in free estradiol:testosterone ratio.

In conclusion, our findings suggest that aging may impair NO/cGMP-mediated relaxation of corporal smooth muscle, leading to impotence. The development of this animal model will facilitate further investigation of the alterations of penile smooth muscle reactivity caused by normal aging process.

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REFERENCES


