Loss of Penile Erectile Response to Intracavernous Injection of Acetylcholine in Castrated Dog

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Castrated dogs showed a slightly weak erectile response to cavernous nerve stimulation compared with normal dogs. Intracavernous injection of acetylcholine induced a dose-dependent increase of intracavernous pressure in normal dogs, however, there was almost no penile response in castrated dogs. Histological study showed a significant higher ratio of collagen tissue/smooth muscle in the corpus cavernosum of castrated dogs, but no difference in acetylcholine-esterase staining and the number of endothelial cells lining the sinusoidal spaces between castrated and normal dogs. These results indicate that the corpus cavernosum of castrated dogs nearly lost responsiveness to acetylcholine probably due to a high ratio of collagen/smooth muscle fibers and that there are different mechanisms for erectile response to nerve stimulation and intracavernous injection of acetylcholine.

castration; acetylcholine; penile erection; corpus cavernosum

The main mechanism of sexual dysfunction in castrated humans and animals has been attributed to loss of sexual arousal, which is dependent on the plasma testosterone level (Michael and Wilson 1974). However, the local effects of castration on the penis are still unclear and controversial. Müller et al. (1988) found a very weak penile erectile response to cavernous nerve stimulation in three castrated dogs. However, Lin et al. (1990) recently reported that castration may not affect penile erectile ability and that a slight reduction of maximal intracavernous pressure in castrated dog was attributed to the concomitant decrease of basal systemic blood pressure.

In the present study, we investigated erectile responses to nerve stimulation and intracavernous injection of acetylcholine (ACh), histological changes in the penis, and plasma testosterone levels in castrated dogs in comparison with those of non castrated normal dogs.

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MATERIALS AND METHODS

Fourteen adult male mongrel dogs weighing 17 to 43 kg were used, six of which were incidentally found to be previously castrated. The time interval of castration was presumed to be 0.5 to 7 years judging by the age of their teeth, and the fact that castration is generally performed at about six months of age in the States.

Animal model

Anesthesia was induced by acepromazine (0.2 mg/kg) and ketamine (10 mg/kg) subcutaneously. Sodium pentobarbital (45–60 mg/hr) was injected intravenously to maintain an adequate level of anesthesia and spontaneous respiration. The animals were placed in the supine position and through a midline abdominal incision the bladder and prostate were exposed. The cavernous nerves were identified posterolaterally to the prostate, and bipolar cuff electrodes (Avery Lab., New York, NY, USA) were placed around them for electrical stimulation. The entire penis was denuded, exposing both corpus cavernosum down to the ishial rami. Two 21-gauge scalp-vein needles were placed into each corpus cavernosum, one proximally for intracavernous pressure (TOP) recording and the other distally for intracavernous injection. Systemic arterial blood pressure was monitored via a 16-gauge cannula in the femoral artery. All fluid-filled lines were connected to Statham pressure transducers and a Grass polygraph for recording.

Nerve stimulation study

To find the minimum voltage required to induce erection which was defined as a pressure more than 80 cmH2O from the basal level by ICP, 0.2, 0.6, 1.2, 1.8, 4.0, 7.0 and 12.0 V of electrical stimulation (1 msec, 20 Hz) were applied to the cavernous nerve for 1 min while ICP and systemic blood pressure were continuously measured. There was on interval of approximately 10 min between each stimulation. We also measured the maximal increase of ICP from the basal level with electrical stimulation, duration of the erecting time when ICP was over 80 cmH2O and the latent period until the ICP peak.

Pharmacological study

To find the minimum dose that effectively increased ICP, 0.1, 0.3, 1, 2, 5, 10, 20, 50, 100, 200, 500 and 700 μg of ACh-chloride (Sigma, St.Louis, MO, USA) was injected intracavernously while recording ICP and systemic pressure.

Testosterone assay

Blood samples were taken from each dog between 8:00 and 9:00 AM during the overnight fast. Samples were immediately centrifuged and stored at −20°C prior to the assay. Plasma testosterone concentration was measured by radioimmunoassay using a specific antibody to testosterone (Peninsula Lab., Belmont, CA, USA).

Histological study

At the end of the study, the penis was removed and both corpus cavernosa were perfused with cold saline for 5 min in order to wash out erythrocytes from the sinusoidal spaces. The cavernous bodies were then removed and cut into two; one block was stored in 10% formaline and stained with hematoxylin-eosin (HE) and Masson-trichrome, the other was immediately frozen in liquid nitrogen and stored at −120°C prior to ACh-esterase staining (Goto et al. 1984). The HE and ACh-esterase stained sections were examined by light microscopy to evaluate the number of endothelial cells lining the sinusoidal spaces, and estimate the amount of ACh-esterase. The light microscopic views of the Masson-trichrome stained tissue were recorded on videotape, and examined by integrative image analyzer, (LA500; PIAS Company, Tokyo). By producing binary images, it was possible to sepa-
rately measure the area of collagen fibers (blue) and smooth muscle (red) of the entire corpus cavernosum, and then calculate the ratio of collagen/smooth muscle.

Values were expressed in terms of mean±s.d. and statistical difference between mean values was determined by unpaired Student's t-test, and was considered significant when $p<0.05$.

**Results**

There was no significant difference in body weight, age and systemic blood-pressure between normal and castrated dogs (Table 1).

**Nerve stimulation study**

The minimum voltage required to induce erection was lower in normal (1.4±1.2 V) than in castrated dogs (3.3±4.3), but the difference was not significant. The maximal increase of ICP was higher in normal (133±29 cmH$_2$O) than in castrated dogs (105±29 cmH$_2$O), although the difference was not significant. The duration of erection and the latent time to the peak ICP in normal dogs were

### Table 1. Characteristics of the subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Normal dog ($n=8$)</th>
<th>Castrated dog ($n=6$)</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>31±4 (24-36)</td>
<td>28±9 (17-43)</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>4.8±2.4 (1-7)</td>
<td>5.1±2.2 (1.5-8)</td>
<td>NS</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>190±47</td>
<td>208±15</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic</td>
<td>120±36</td>
<td>118±5</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are expressed as mean±s.d.

NS, not significant.

### Table 2. Penile erectile response induced by cavernous nerve stimulation in normal dogs compared with castrated dogs

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Normal dog ($n=8$)</th>
<th>Castrated dog ($n=6$)</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum voltage to induce erection</td>
<td>1.4±1.2 (0.2-4.0)</td>
<td>3.3±4.3 (0.6-12)</td>
<td>NS</td>
</tr>
<tr>
<td>Increase of ICP (cmH$_2$O)</td>
<td>133±29</td>
<td>105±29</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of erection (ICP &gt; 80 cmH$_2$O) (sec)</td>
<td>93±42</td>
<td>72±45</td>
<td>NS</td>
</tr>
<tr>
<td>Latent period until the peak of ICP (sec)</td>
<td>54±14</td>
<td>56±13</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are expressed as mean±s.d.

ICP, intracavernous pressure; NS, not significant.
almost the same as those of castrated dogs (Table 2).

**Pharmacological study**

Intracavernous injection of ACh induced a dose-dependent increase of ICP in normal dogs, however, in castrated dogs a dose greater than 200 μg ACh induced a transient systemic hypotension with a slight increase of ICP (Fig. 1). The minimum dose of ACh required to increase ICP was $15 \pm 20 \, \mu g$ in normal dogs, and $383 \pm 223 \, \mu g$ in castrated dogs; the difference was significant ($p < 0.01$). The increase of ICP induced by the minimum dose of ACh was not different significantly between normal and castrated dogs (Table 3).

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**Fig. 1.** Intracavernous injection study with ACh. ACh induced a transient, dose-response increase of ICP with no change of BP in normal dog, but little effect on ICP with systemic hypotension in castrated dog.

**Table 3.** Results of intracavernous injection study with ACh and histological study of penis in normal dogs compared with castrated dogs

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Normal (n)</th>
<th>Castrated (n)</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum dose of ACh to increase ICP ($\mu g$)</td>
<td>$15 \pm 20$ (8)</td>
<td>$383 \pm 223$ (6)</td>
<td>$p &lt; 0.005$</td>
</tr>
<tr>
<td>Increase of ICP ($cm H_2O$)</td>
<td>$14 \pm 9.3$ (8)</td>
<td>$7.7 \pm 8.8$ (6)</td>
<td>NS</td>
</tr>
<tr>
<td>Collagen/smooth muscle*</td>
<td>$2.0 \pm 0.5$ (5)</td>
<td>$4.0 \pm 1.7$ (5)</td>
<td>$p &lt; 0.05$</td>
</tr>
<tr>
<td>ACh-esterase staining</td>
<td>$+ \sim #$ (6)</td>
<td>$+ \sim #$ (4)</td>
<td></td>
</tr>
<tr>
<td>Endothelial cells</td>
<td>$+ \sim #$ (6)</td>
<td>$+ \sim #$ (6)</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D.

*Ratio of collagen tissue per smooth muscle in cavernous body.

ACh, acetylcholine; ICP, intracavernous pressure.
Histological study

Masson-trichrome staining revealed more red than blue staining in the cavernous body of normal dogs, and the ratio of collagen fiber/smooth muscle was $2.0 \pm 0.5$, which was significantly different from that of castrated dogs ($4.0 \pm 1.7$) (Fig. 2). ACh-esterase staining showed brown-black deposits within the smooth muscle trabeculae of the cavernous body and arteries of the penis. However, there was so much individual variation that no significant difference between normal and castrated dogs was found. The density of endothelial cells lining the sinusoids, evaluated by HE-staining, also showed so much individual variation that no significance was found between the two groups (Table 3).

Plasma testosterone concentration

The level of plasma testosterone measured in six normal dogs varied widely from 110 to 3,000 pg/ml, but in four castrated dogs it could not be detected at all (less than 100 pg/ml).

Discussion

Poor erectile response to nerve stimulation in castrated dogs has already been recognized by some investigators (Müller et al. 1988; Lin et al. 1990). Müller supposed that it may be a result of dysfunction of venous occlusion and/or incomplete relaxation of sinusoidal smooth muscle. However, Lin attributed it to decreased basal systemic blood pressure, and not to the penis itself.

According to our present results, castrated dogs showed a greatly diminished erectile response to ACh injected intracavernously, however, the erectile response to nerve stimulation did not differ much from that of normal dogs. This means that there are different mechanisms for erectile responses induced by nerve stimu-
lation and intracavernous injection of ACh. It can be speculated that the amount of neurotransmitter released by nerve stimulation is so much that the resulting erectile activity may overcome the reduced amount of smooth muscle in the corpus cavernosum of castrated dogs. The high ratio of collagen/smooth muscle in castrated dogs may be responsible for the poor response to ACh injected intracavernously but not play so much role on the response to nerve stimulation.

Another possibility for the poor response to ACh in castrated dogs is a hormonal influence; steroid hormones are known to directly influence the levels of neurotransmitter receptors and the decreased muscarinic cholinergic receptor (MChR) density following castration appears to be due to a castration-associated down regulation of MChR (Bleisch et al. 1984; Ellen et al. 1985). Thus, the poor response to ACh in castrated dogs might be due to down regulation of MChR on endothelial cells of the sinusoidal spaces.

At present, we have no data to explain why castrated dogs have a high ratio of collagen fiber/smooth muscle in spite of the same age as control dogs, however, we can speculate that during the several months and years following castration, loss of sexual activity may induce disuse atrophy of erectile muscle including cavernous smooth muscle. Another possibility is a direct effect of testosterone on cavernous smooth muscle, since testosterone is known to act as a promotor of growth of striated muscle, including bulbospongious muscle (Hughes et al. 1983).

We also found a similar number of endothelial cells lining the sinusoids and ACh-esterase staining did not differ between castrated and normal dogs.

ACh is now known to cause relaxation of smooth muscle through endothelium dependent relaxing factor (EDRF) when injected intracavernously (Tejada et al. 1988). Therefore, not only the number of endothelial cells lining the sinusoids, but also the amount of EDRF excreted from the cells, should be examined in order to evaluate the mechanism of the poor response to intracavernous injection of ACh.

In conclusion, castrated dogs had a high ratio of collagen fiber to smooth muscle in the cavernous body, and this may be responsible for the poor response to ACh when injected intracavernously.

References

5) Lin, S.N., Yu, P.C., Huang, J.K., Yang, M.C.M., Chang, L.S., Chai, C.Y. & Kuo, J.S.
Castration may not affect the penile erection ability in terms of peripheral neurocavernous mechanism in dogs. *J. Urol.*, **143**, 172–174.

