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Possible Role for Acetylcholine as a Neurotransmitter in Canine Penile Erection

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Key Words. Penile erection · Acetylcholine · Muscarinic · Nicotinic · Acetylcholinesterase · Neurotransmitter · Neurostimulation

Abstract. In 15 adult dogs, the possible role of acetylcholine as a parasympathetic neurotransmitter in canine penile erection was investigated. Intracavernous injection of increasing dosages of acetylcholine (0.1–100 µg) induced a dose-dependent erectile response with increased arterial flow, cavernous smooth muscle relaxation, and venous occlusion. This erectile response was completely abolished after muscarinic blockade by intracavernous injection of 0.1 mg atropine. After cavernous nerve stimulation, atropine injection significantly reduced the pudendal arterial flow (by 25%) and likewise caused a significant reduction in cavernous outflow restriction. Histologic staining showed acetylcholinesterase-positive fibers around the cavernous arteries and within the cavernous erectile tissue.

By means of descriptive anatomy, Kölliker [18] postulated in 1852 that penile erection is due to arterial relaxation with subsequently increased arterial flow, cavernous smooth muscle relaxation and restriction of venous drainage. In 1863, Eckard [10] was the first to examine the phenomenon of canine penile erection functionally by galvanic stimulation of the nervi erigentes. He showed these nerves to be parasympathetic, emerging from S1 to S3. Since then, these findings have been reproduced in different species [1, 2, 7, 9, 14, 19, 20, 22, 24].

According to the classic theory of autonomic parasympathetic neurotransmission [11], acetylcholine (ACh) has been postulated as the neurotransmitter for penile erection. Over a century ago, Anrep and Cybulsky [3] reported that erection is atropine-resistant and Nikolsky [22] found that erection induced by stimulation of the nervi erigentes could be abolished by atropine. Nevertheless, the question of the atropine-sensitivity of erection remains, with *in vivo* studies yielding results that are both contradictory [5, 7, 14, 27] and confirmatory [9, 24].

In vitro findings have also been conflicting: no effect [4, 8]; relaxation [6, 13] or contraction [17] of the cavernous smooth muscle, and inconsistent effects [1].

The aim of this study was to elucidate the role of ACh in canine penile erection *in vivo*.

Materials and Methods

In 15 adult male mongrel dogs (21–34.5 kg), anesthesia was induced by subcutaneous injection of acepromazine (5 mg/kg body weight) and ketamine (0.5 mg/kg body weight) and maintained by intravenous infusion of sodium pentobarbital (approximately 1 mg/kg body weight/h). Systemic blood pressure was monitored via a cannula in the femoral artery.

With the animal in the supine position, the abdominal cavity was exposed via a mid-line incision. An ultrasonic flow probe (Transsonic Systems Inc., N.Y.) was placed around the right internal pudendal artery to measure the arterial blood flow to the penis. In 9 dogs (No. 7–15), the cavernous nerves were identified by neurostimulation with a prick electrode (Avery Laboratories), caudal to their branching from the pelvic nerve. A bipolar cuff electrode (Avery Laboratories) was placed around the cavernous nerve bundles bilaterally. Penile erection was induced with an Avery Stimulator (0.6–1.2 V, 20 Hz; pulse duration 1 ms; stimulation time 1 min).

The penis was dissected in all dogs, exposing both corpora cavernosa from the pubic bone to the prepuce. For intracavernous pressure recording (Grass Polygraph, Model 7), a 21-gauge scalp-vein

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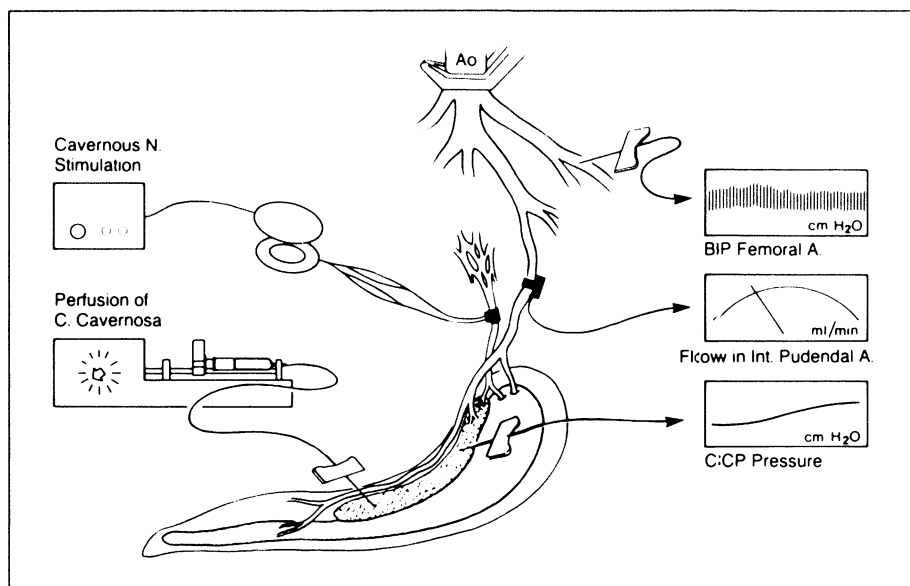


Fig. 1. Apparatus for protocol. Erection was induced by cavernous nerve stimulation or intracavernous injection of ACh. Arterial response was measured by Doppler analysis of the distal pudendal artery. Venous occlusion was measured by cavernous perfusion with clamped aorta, and intracavernous pressure was recorded via a needle inserted into the cavernous body.

Table 1. Canine erectile response to intracavernous injection of acetylcholine (ACh)

ACh µg	Pudendal arterial flow ¹		Intracavernous pressure ²	
	maximum increase ml/min	duration of increase ³ s	maximum increase cm H ₂ O	duration of increase s
0.1	2-4 (2.8)	55-90 (77)	28-60 (49)	50-120 (96)
1	3.5-7 (4.4)	90-300 (141)	45-112 (72)	90-310 (169)
10	4-7 (5.8)	165-310 (229)	52-124 (91)	180-360 (245)
100	5-13 (11)	385-610 (452)	104-144 (127)	360-540 (443)

All values depict the range, with the mean in parentheses.

¹ Baseline pudendal arterial flow = 6.4 ml/min (4-10 ml/min).

² Baseline intracavernous pressure = 20 cm H₂O (12-32 cm H₂O).

³ Values represent entire duration of increase, both maximal and moderate elevations, before return to baseline.

needle was inserted proximally into each cavernous body and connected to a Statham transducer (Model P23). To evaluate the cavernous outflow system without the influence of arterial flow, the aorta was dissected for occlusion with a Satinsky clamp proximally to the branching of the external iliac arteries. For continuous cavernous perfusion, a 19-gauge scalp-vein needle was inserted distally into each cavernous body and connected to a Harvard perfusion pump. Anticoagulation was achieved by 1,000 U sodium heparin intravenously and maintained with 50 U/h (fig. 1).

Intracavernous Injection of ACh

Increasing dosages of ACh (0.1, 1 [5×10^{-9} mol], 10 and 100 µg; Miocholine, Cooper Vision, Puerto Rico) were injected into the right cavernous body, with an interval of 15 min between each injection. To verify reproducibility, the injection protocol was then repeated on the left cavernous body.

Venous Study after Intracavernous Injection of ACh

To study the cavernous outflow, the aorta was clamped and saline solution (37°C) was perfused intracavernously at constant rates of 3.8, 7.6, 15.3 and 26.6 ml/min. Each perfusion was delivered at 1, 3 and 5 min after clamping the aorta, with a 15-min interval allowed before increasing the perfusion rate. Perfusions lasted for 1 min or until the intracavernous pressure surpassed 180 cm H₂O (out of scale). The perfusion study was then repeated with the intracavernous injection of 100 µg ACh 30 s after clamping the aorta.

Intracavernous Injection of ACh after Muscarinic Blockade

After intracavernous injection of 0.1 mg atropine (1.44×10^{-7} mol), increasing dosages of ACh (0.1, 1, 10 and 100 µg) were injected intracavernously with an interval of 3 min between each injection.

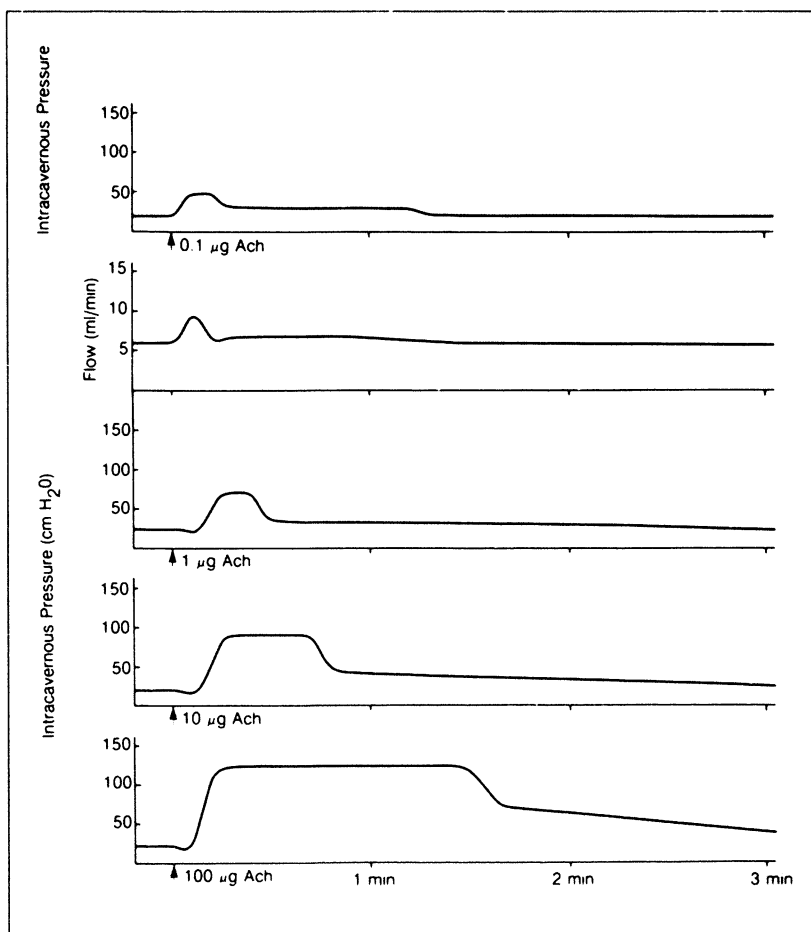


Fig. 2. Erectile responses to intracavernous injection of ACh at different doses (0.1–100 μg). For 1 μg , the flow response is also shown.

Effect of Muscarinic Blockade on Neurostimulation-Induced Erection

To study the effect of muscarinic blockade on neurostimulated erection, 0.1 mg atropine was injected in dogs 7–12 after five control stimulations with recordings of internal pudendal arterial and intracavernous pressure. One minute after the injection of atropine, five more erections were induced by neurostimulation. The interval between each neurostimulation was 5 min.

Effect of Muscarinic Blockade on Neurostimulation-Induced Cavernous Outflow Occlusion

To study the influence of muscarinic blockade on the cavernous outflow in neurostimulated erections, a venous study was performed in dogs 13–15. After clamping the aorta, the cavernous nerve was stimulated at 30 s; at 50 s, the cavernous body was perfused with saline solution (37 °C) at the rate of 3.8 ml/min until the intracavernous pressure reached 180 cm H₂O. This control study was repeated four times. It was then performed five times after intracavernous injection of 0.1 mg atropine.

Histologic Staining for Acetylcholinesterase

At the end of the study, the aorta was clamped in 7 dogs (No. 1–7) and the distal aorta was perfused with 2 liters of saline to wash

out the erythrocytes from the cavernous spaces. The cavernous bodies were then removed, frozen in liquid nitrogen and processed for acetylcholinesterase (AChE) staining [12].

Analysis of Data

Statistical analysis was performed with the Student's *t* test.

Results

Intracavernous Injection of ACh

The intracavernous injection of ACh into the right cavernous body led to an immediate increase in flow in the right pudendal artery, followed by a moderate elevation in flow throughout erection. The rise in cavernous pressure followed the arterial flow increase after a delay of 8–14 s. The initial strong increase in intracavernous pressure had a dose-dependent duration (from a mean of 14 s after 0.1 μg ACh to a mean of 84 s after 100 μg ACh; table 1) and then rebounded to moderately elevated levels (fig. 2). The intracavernous injection of ACh into the

left cavernous body induced comparable responses in the intracavernous pressure, but no significant changes in arterial flow within the right pudendal artery.

Injections of ACh ($\leq 10 \mu\text{g}$) had no effect on systemic blood pressure. In 5 of 6 dogs, the injection of $100 \mu\text{g}$ lowered the blood pressure from a mean of 160/130 to 120/70 mm Hg for 15–20 s.

Venous Study after Intracavernous Injection of ACh

After clamping the aorta, saline perfusion alone of the cavernous body induced a rise in intracavernous pressure that plateaued after 11–16 s (fig. 3). The mean pressure at this plateau was 32 cm H₂O at a flow rate of 3.8 ml/min, 45 cm H₂O at 7.6 ml/min, 65 cm H₂O at 15.3 ml/min and 87 cm H₂O at 26.6 ml/min.

After intracavernous injection of $100 \mu\text{g}$ ACh, the first perfusion (1 min after clamping the aorta) increased the intracavernous pressure to above 180 cm H₂O at all flow rates in all dogs, indicating cavernous outflow occlusion by ACh. At the second perfusion (min 3), venous occlusion (intracavernous pressure > 180 cm H₂O within 60 s of perfusion) occurred in all dogs with perfusion rates of 15.3 and 26.6 ml/min. At the third perfusion (min 5), venous occlusion was found only in 1 dog in response to the perfusion rate of 26.6 ml/min.

Intracavernous Injection of ACh after Muscarinic Blockade

After the intracavernous injection of 0.1 mg atropine, ACh had no effect on pudendal arterial flow or intracavernous pressure, regardless of the dosage.

Effect of Muscarinic Blockade on Neurostimulation-Induced Erection

Control neurostimulation of the right cavernous nerve induced a full erection in all 6 dogs, with a mean maximal intracavernous pressure increase of 117.9 cm H₂O (range 88–140) above baseline (table 2). A pressure increase of 60 cm H₂O was reached after a mean of 18.5 s (range 10–22) after the beginning of stimulation; an increase of 80 cm H₂O required a mean of 31.2 s (range 14–76). The mean maximal flow increase after neurostimulation was 33.5 ml/min (range 30–38).

After muscarinic blockade, the mean maximal intracavernous pressure increase was 117.9 cm H₂O (range 100–128) above baseline. A pressure increase of 60 cm H₂O was reached after a mean of 27.3 s (range 16–36) from the beginning of stimulation. At the end of the study (i.e. at the fifth stimulation), the time required to attain this level was 39.2 s (range 24–72). The mean

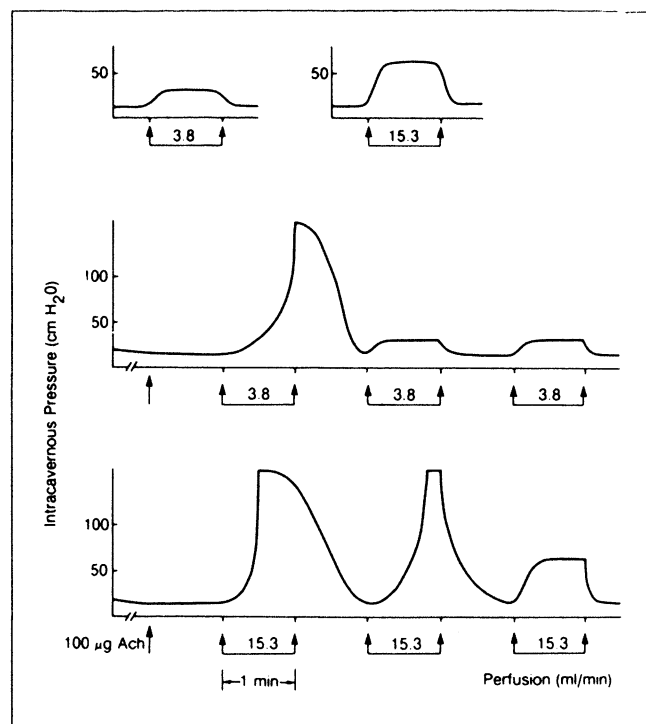


Fig. 3. Top: With the aorta clamped, saline perfusion showed no venous occlusion. Middle: After $100 \mu\text{g}$ ACh intracavernously, venous occlusion was found 1 min after injection of a perfusion rate of 3.8 ml/min. Bottom: 3 min after intracavernous injection of $100 \mu\text{g}$ ACh, venous occlusion could be induced by perfusion rates of 15.3 ml/min and greater.

maximal flow increase after neurostimulation was 24.9 ml/min (range 21–32).

The reduction of the maximal flow increase by 25% after atropine was statistically significant ($p < 0.001$), as was the prolongation after atropine of the time required for the intracavernous pressure to attain a landmark level (40, 60, 80 cm H₂O). The maximal intracavernous pressure levels were not significantly different before and after atropine ($p = 0.99$). Likewise, there were no significant differences between the times required for the intracavernous pressure to drop back to landmark levels before and after atropine.

Effect of Muscarinic Blockade on Neurostimulation-Induced Cavernous Outflow Occlusion

After clamping the aorta, neurostimulation and subsequent cavernous perfusion of 3.8 ml/min, the intracavernous pressure reached 180 cm H₂O after a mean perfusion time of 24.5 ± 1.9 s. After muscarinic blockade, this time increased to 34.4 ± 3.9 s ($p < 0.001$).



Fig. 4. Histologic staining revealed fibers positive for AChE around the cavernous artery (a) and within the cavernous smooth muscle (b). Magnification $\times 400$ and $\times 100$, respectively, before reduction.

Table 2. Effect of intracavernous injection of 0.1 mg atropine on neurostimulation-induced canine penile erection

	Pudendal arterial flow ml/min	Time (s) needed to reach intracavernous pressure increase			Maximal intracavernous pressure cm H ₂ O
		+40 cm H ₂ O	+60 cm H ₂ O	+80 cm H ₂ O	
Before atropine	33.5 ± 3.9	15.4 ± 3.0	18.5 ± 4.0	22.6 ± 4.9	117.9 ± 12.4
After atropine	24.9 ± 2.8*	22.5 ± 4.3*	27.3 ± 5.4*	31.3 ± 6.1*	117.9 ± 6.7**

* p < 0.001; ** p > 0.05.

Histologic Staining for AChE

Fibers positive for AChE were found in all dogs around the cavernous artery, within nerve bundles near the cavernous arteries, and within the cavernous erectile tissue (fig. 4).

Discussion

Our findings show that intracavernous injection of ACh in the canine induces a dose-dependent erectile response (e.g. increased arterial flow, relaxation of the cavernous smooth muscles, and venous occlusion). This response was completely abolished after muscarinic blockade by intracavernous atropine. Thus, the induction of erection by intracavernous ACh represents a muscarinic effect in the canine model; in contrast, in men and monkeys intracavernous ACh also has a nicotinic (ganglionic) effect both *in vitro* [1] and *in vivo* [25]. In the present study, the neurostimulation-induced erection was only modulated, but was not abolished, by the intracavernous injection of atropine. A significant reduction in arterial flow delayed the time required for the intracavernous pressure to attain maximal levels. The venous study during neurostimulation showed reduced cavernous outflow restriction after atropine, indicating less complete cavernous smooth muscle relaxation [15] than during neurostimulation without previous muscarinic blockade.

The above findings suggest that the role of ACh in canine penile erection resides in its effect on the cavernous artery and cavernous smooth muscle. This is supported by the histologic studies showing AChE-positive staining around the cavernous artery and within the cavernous erectile tissue.

Our results are in accordance with those of other *in vivo* studies; Valji and Bookstein [26] have described penile erection in the dog after intracavernous injection of ACh; Dorr and Brody [9] and Andersson et al. [2] have shown that atropine significantly reduces the canine erectile response to neurostimulation; and McConnell et al. [21] and Shirai et al. [23] have reported AChE-positive fibers around the cavernous artery and within the cavernous erectile tissue in men, monkeys, cats, rabbits and dogs.

In contrast, no erectile response has been observed after intra-aortic injection of ACh [14]. This may be due to the immediate inactivation of ACh by AChE in the blood, to the pronounced lowering of the systemic blood pressure in response to high doses of ACh, or to the fact

that these investigators recorded the pressure in the glands rather than in the cavernous bodies. In another study, Carati et al. [7] administered high doses of atropine (1 mg/kg body weight), but found no effect on the erectile response to pelvic nerve stimulation. We think that this may be due to the systemic application of atropine. The muscarinic blockade for erection may be more complete, even after much smaller doses, when atropine is injected directly into the cavernous body instead of into the systemic circulation.

The canine cavernous erectile tissue is reportedly relaxed by ACh [6], as are human cavernous tissue strips [13]. These two *in vitro* studies supporting our *in vivo* findings are at variance with the *in vitro* results of other investigators, who have reported both no effect of ACh on human cavernous smooth muscle strips [4, 21] and inconsistent effects [1]. In muscle strips of different species, about a third showed contraction after application of ACh [17]. These differences may have several explanations: to prove a relaxing effect, the smooth muscle must first be contracted by electrical or pharmacologic means. In those studies where this was done before the application of ACh, a relaxing effect was shown. Inconsistent effects may be due to simultaneous nicotinic and muscarinic stimulation in the cavernous tissue of monkeys and men. Finally, the action of ACh may depend on the integrity of the cavernous endothelium, releasing the endothelium-releasing factor that is responsible for the effect of ACh on erection [Goldstein I., personal communication]. A destruction of the cavernous endothelium during tissue preparation could prevent any subsequent effect of ACh on the smooth muscle. In conclusion, our *in vivo* results, together with the histologic findings, suggest a possible role for ACh in canine penile erection. Further studies are needed to reveal additional neurotransmitters responsible for the erectile response to neurostimulation after muscarinic blockade.

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