JOURAA
ISSN 0022-5347
Volume 141 June 1989 Number 6

The Journal of UROLOGY®

FOUNDED IN 1917 BY HUGH HAMPTON YOUNG

Official Journal of the American Urological Association, Inc.

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0022-5347/89/1416-1444$02.00/0
THE JOURNAL OF UROLOGY
Vol. 141, Jun
Printed in U.S.A

ACETYLCHOLINE AS A POSSIBLE NEUROTRANSMITTER IN PENILE ERECTION

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ABSTRACT

We investigated the erectile response to intracavernous injection of increasing doses of acetylcholine (0.5 to 500 μg.) in 10 monkeys. To differentiate between nicotinic (ganglionic) and muscarinic (parasympathetic postganglionic) effects, acetylcholine was likewise administered after 1.6 mg. trimethaphan camsylate and 0.1 mg. atropine, alone or sequentially. Erections were induced by cavernous nerve stimulation before and after atropine.

Acetylcholine induced a dose-dependent, triphasic erectile response: a first tumescence phase followed by contraction and a subsequent second phase of tumescence. Atropine reduced but did not abolish the erectile response to acetylcholine: attainment of maximal intracavernous pressure after neurostimulation was both delayed and reduced (mean 25 cm. H_2O). Only after combined nicotinic and muscarinic blockade was the erectile response to acetylcholine completely abolished. Histologic staining for acetylcholinesterase in five additional monkeys that had not received acetylcholine showed dense staining within the cavernous erectile tissue and around the cavernous arteries.

Our data suggest that acetylcholine is a possible neurotransmitter for penile erection in monkeys. (*J. Urol., 141: 1444-1448, 1989*)

Since the report of Eckhardt in 1863 that galvanic stimulation of pelvic parasympathetic nerves induces penile erection in the dog,[1] many studies have confirmed these findings in different species.[2-7] Based on the classic theory of autonomic neurotransmission, the muscarinic neurotransmitter was believed to be acetylcholine (ACh).[8] However, penile erection has been shown to be atropine-resistant,[9,10] and organ bath studies of the effect of ACh on cavernous smooth muscle have been inconclusive. In vitro, all possible responses to ACh have been observed: no effect;[11,12] inconsistent effects;[13] and both contraction[14] and relaxation[15,16] of the cavernous smooth muscle. Our aim was to examine in vivo a possible role of ACh as a postganglionic parasympathetic neurotransmitter for penile erection.

MATERIALS AND METHODS

Ten pigtail monkeys, weighing 4.3 to 10.5 kg., were used. After adequate anesthesia with ketamine (30 mg./kg. bodyweight i.m.), the monkeys were placed in the supine position. Under sterile conditions, one 21-gauge scalp-vein needle was placed in each distal right and left cavernous body. One needle was connected to a Statham transducer (model P23 BC) for intracavernous pressure recording (Grass Polygraph; model 7); the other served for intracavernous injection or perfusion. Penile tumescence was visually monitored by at least two investigators and recorded. The flow through the cavernous arteries was measured in four monkeys (No. 7-10) by duplex ultrasound.[17] Pulse and blood pressure were monitored closely with the aid of a Doppler probe (Parks Medical Electronics) on the radial artery and a pediatric blood pressure cuff (fig. 1).

Intracavernous injection of ACh. All monkeys received doses of 0.5, 1 (5×10^{-9} mol.), 10, 100 and 500 μg. ACh (Miocholine, Cooper Vision, Puerto Rico) by intracavernous injection. The effects of each dose were allowed to subside and a 10 to 15 minute waiting period intervened before the next injection. To verify reproducibility, all injections were given twice.

Intracavernous injection of ACh after muscarinic blockade. The above injection protocol was followed after intracavernous injection of 0.1 mg. atropine (1.4×10^{-7} mol.) (Elkins-Sim Inc., Cherry Hill, N.Y.). This dosage was chosen after 0.01 mg. intracavernously proved to be sufficient for complete muscarinic blockade in dogs (unpublished data). Although the size of the cavernous bodies in monkeys and dogs is comparable, a ten-fold increase (0.1 mg.) was selected to ensure complete local muscarinic blockade.

Erections induced by neurostimulation before and after atropine. In monkeys 1-6, cuff electrodes were placed around the cavernous nerve as previously described.[18] After three erections had been induced by neurostimulation (20 Hz, six to eight V, stimulation time = one minute), 0.1 mg. atropine was injected intracavernously and neurostimulation was repeated five times with an eight-minute interval between each stimulation.

Intracavernous injection of ACh after nicotinic blockade. To investigate the effect of ACh after nicotinic blockade, the six monkeys with implanted electrodes were given an intracavernous injection of 1.6 mg. trimethaphan camsylate (Roche Laboratories, Nutley, N.J.), the lowest dosage shown to block neurostimulation-induced erections in all monkeys. The drug's intrinsic activity resulted in penile tumescence with venous occlusion for 180 to 290 seconds (mean 235 seconds). However, during this period and for the next 180 to 200 seconds, the erectile response to neurostimulation was completely abolished. Acetylcholine (0.5 mg.) was injected intracavernously after trimethaphan camsylate's intrinsic activity had subsided (by direct vasodilation and smooth muscle relaxation) but during maintenance of nicotinic blockade.

Injection of trimethaphan camsylate and ACh was repeated after 0.1 mg. atropine.

The effect of ACh on the cavernous outflow. In five monkeys, a flow probe was placed around the pudendal artery and the aorta was dissected for intermittent clamping with a Satinsky clamp. With the aorta clamped and the intracavernous pressure at baseline (mean 11 cm. H_2O), the penis was perfused with

Accepted for publication December 15, 1988.
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Supported by a grant from Deutsche Forschungsgemeinschaft.

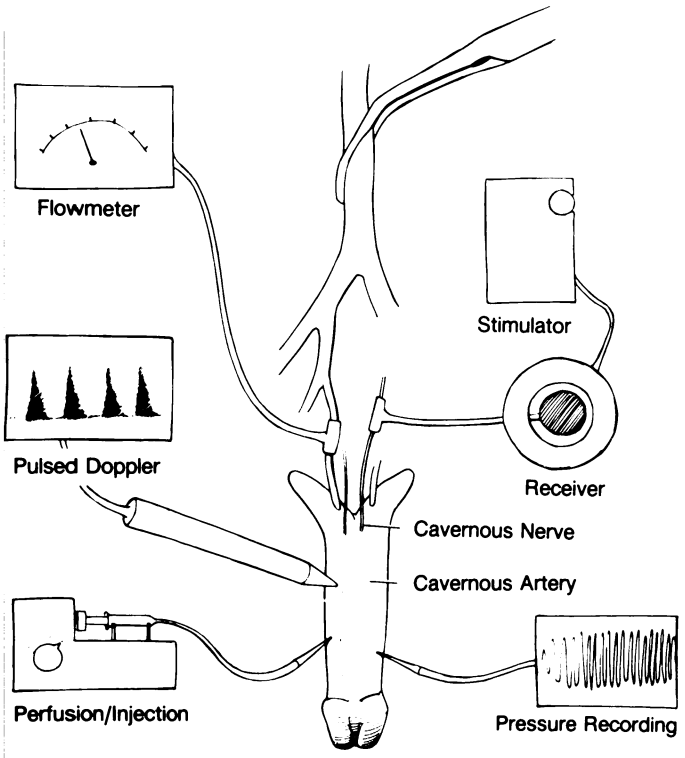

FIG. 1. Erection was induced in monkeys by cavernous nerve stimulation or intracavernous injection. Arterial response was measured by Duplex ultrasound or by flow probe placed around pudendal artery, or both. Intracavernous pressure response was recorded via needle inserted in cavernous body.

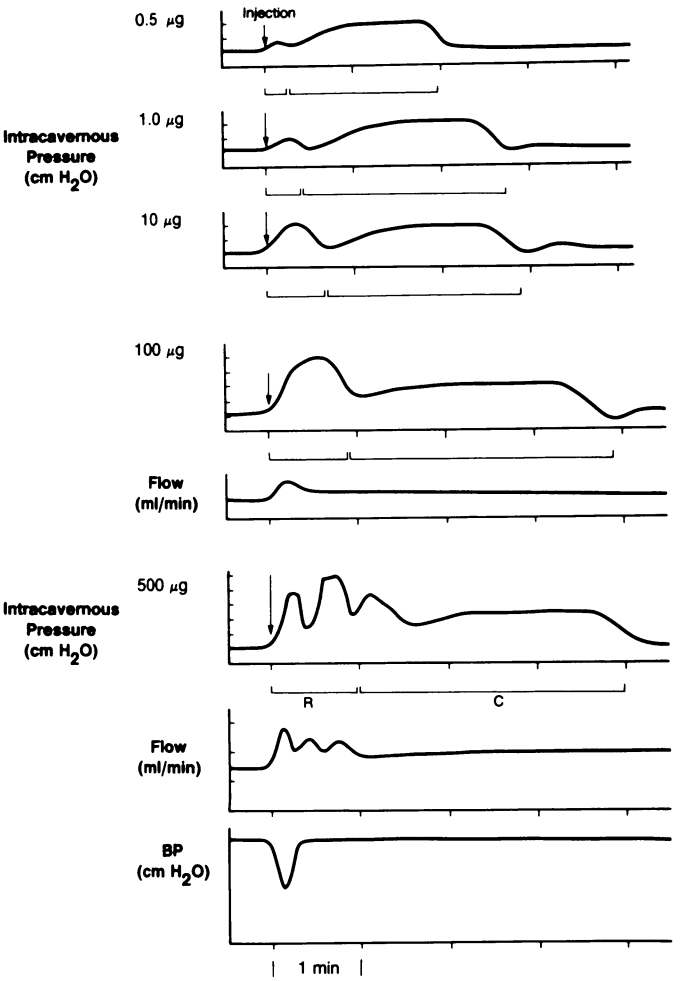

FIG. 2. Erectile responses to intracavernous injection of ACh at different doses.

saline (37C) at increasing rates (3.8, 7.6, 15.3 and 26.6 ml./min.) before and after 0.5 mg. ACh intracavernously. When the perfusion had continued for 60 seconds or the intracavernous pressure surpassed 200 cm. H_2O, it was stopped. After intervals of 90, 60 and 60 seconds the penis was re-perfused. The clamp on the aorta was then released.

Histologic staining for acetylcholinesterase. In five monkeys in which no neurotransmitter studies had been performed (i.e. animals to be sacrificed by other departments at our institution), the aorta was clamped and the distal aorta was perfused with two liters of saline to wash the red blood cells from the penile tissue. The penis was then immediately frozen and stained for acetylcholinesterase (AChE).[19]

RESULTS

Intracavernous injection of ACh. Intracavernous injection of ACh induced a dose-dependent increase in penile tumescence, intracavernous pressure, and flow through the internal pudendal artery (fig. 2). The lowest dosage that induced penile tumescence was 0.5 μg. in three monkeys, one μg. in six, and 10 μg. in one. The first observable change was an increase in arterial flow, followed by penile tumescence, and then, after some seconds, by an increase in intracavernous pressure. The duration of tumescence increased with increasing dosages: from a mean of nine seconds (zero to 14 seconds) after 0.5 μg. to a mean of 82 seconds (60 to 96) after 500 μg. ACh.

A characteristic tri-phasic response was seen in seven monkeys with dosages up to 100 μg. and two monkeys up to 500 μg. Acetylcholine first induced penile tumescence and a rise in intracavernous pressure. This was followed by penile contraction and a plateau in intracavernous pressure at a level above baseline (fig. 2). During penile contraction, the arterial flow within the cavernous artery was markedly greater than in the flaccid state. This contraction was followed by another increase in tumescence and intracavernous pressure, both less dramatic than those in the first phase (table 1).

A multi-peaked, wave-shaped intracavernous pressure response was seen after 100 μg. in three monkeys and after 500 μg. in four (fig. 2). In the majority, 0.5 μg. ACh caused the systemic blood pressure to fall below 40 mm. Hg for about 20 to 40 seconds.

Intracavernous injection of ACh after muscarinic blockade by atropine. Intracavernous injection of 0.1 mg. atropine was not followed by changes in heart rate or systemic blood pressure. It induced an increase in the arterial flow of brief duration (five to 10 seconds) and slight tumescence lasting 10 to 25 seconds. The subsequent response to ACh remained dose-dependent: 0.5 and one μg. did not result in tumescence, but only in penile contraction with a subsequent intracavernous pressure increase (table 1). Compared with the effect induced by ACh alone, the response to ACh after atropine injection was markedly different: the duration of tumescence and the increase in intracavernous pressure and arterial flow were less; the contraction phase was more pronounced (high intracavernous pressure) and more prolonged; no second tumescence phase occurred. In the monkeys with wave-shaped erectile responses to 100 and 500 μg. ACh, the same doses after atropine induced a continuous, sustained erectile response.

Erection induced by neurostimulation before and after atropine. In all six monkeys with electrodes around the cavernous nerve, electrical stimulation induced a reproducible full erection. After 0.1 mg. atropine, the first neurostimulation produced the same full erection as before. In the subsequent neurostimulated erections, the phase until attainment of maximal intracavernous pressure was prolonged by 10 to 34% (mean 28%; a mean delay of four seconds; $p < 0.001$ [Student's t test]) and

TABLE 1. *Erectile response to acetylcholine before and after atropine*

ACh (μg.)	First Tumescence		Contraction		Second Tumescence	
	Duration (min.)	Pressure* (cm. H_2O)	Duration (min.)	Pressure (cm. H_2O)	Duration (min.)	Pressure (cm. H_2O)
0.5	15	35†	90	60	60	32
1	24	40	120	60	60	40
10	32	65	120	60	70	40
100	54	105	150	60	90	25
500	84	120	180	75	90	25
After 0.1 mg. atropine						
0.5	-	-	40	60	-	-
1	-	-	62	60	-	-
10	14	42	165	90	-	-
100	22	60	210	100	-	-
500	30	75	260	100	-	-

* Intracavernous pressure.
† Numbers represent mean values for all monkeys that responded (3 to 0.5 μg., 6 to 1.0 μg. 7 to 10 μg., and all 10 to 100 and 500 μg).

the full erection pressure was decreased by 12 to 20% (mean 16% or 25 cm. H_2O; $p < 0.05$). The detumescence phase was unchanged. The immediate, strong increase in flow within the internal pudendal artery at cavernous nerve stimulation was decreased by a mean of 29%.

Intracavernous injection of ACh after nicotinic blockade by trimethaphan camsylate. Intracavernous injection of trimethaphan camsylate was followed by a mean reduction in systemic blood pressure of 12 cm. H_2O for two to three minutes. After nicotinic blockade, intracavernous injection of 0.5 mg. ACh induced a similar erection to the one before trimethaphan camsylate, but attainment of maximal intracavernous pressure was delayed by a mean of nine seconds.

The injection of 0.5 mg. ACh after 0.1 mg. atropine and nicotinic blockade induced slight penile tumescence in one of the six monkeys, but no tumescence or increase in intracavernous pressure in five.

The effect of ACh on the cavernous outflow. With the aorta clamped, saline perfusion after ACh injection resulted in a perfusion-dependent plateau in intracavernous pressure without penile tumescence. Immediately after injection of 0.5 mg. ACh, perfusion rates of 3.8 ml./min. and higher induced full penile erection (intracavernous pressure > 200 cm. H_2O). Reperfusion after 90 seconds produced the same result. However, with the third perfusion, flow rates up to 26.6 ml./min. did not induce any tumescence, although, at the fourth perfusion, flow rates ≥ 15.3 ml./min. again induced full erection (fig. 3). Thus, this perfusion study shows that intracavernous injection of ACh induced cavernous smooth muscle relaxation and subsequent cavernous outflow occlusion during the first two perfusions, but no cavernous occlusion (or smooth muscle relaxation) during the third. With the fourth perfusion, cavernous occlusion was again demonstrated, although it was not as pronounced as immediately after ACh injection (probably owing to a lesser degree of smooth muscle relaxation). Thus, the perfusion study confirmed the above-described findings of a triphasic erectile response to intracavernous ACh.

Histologic findings. In the histologic sections from the five monkeys, dense staining for acetylcholinesterase was seen around the cavernous artery, within nerves near the cavernous artery, and within the cavernous erectile tissues. No staining was found in the tunica albuginea (fig. 4).

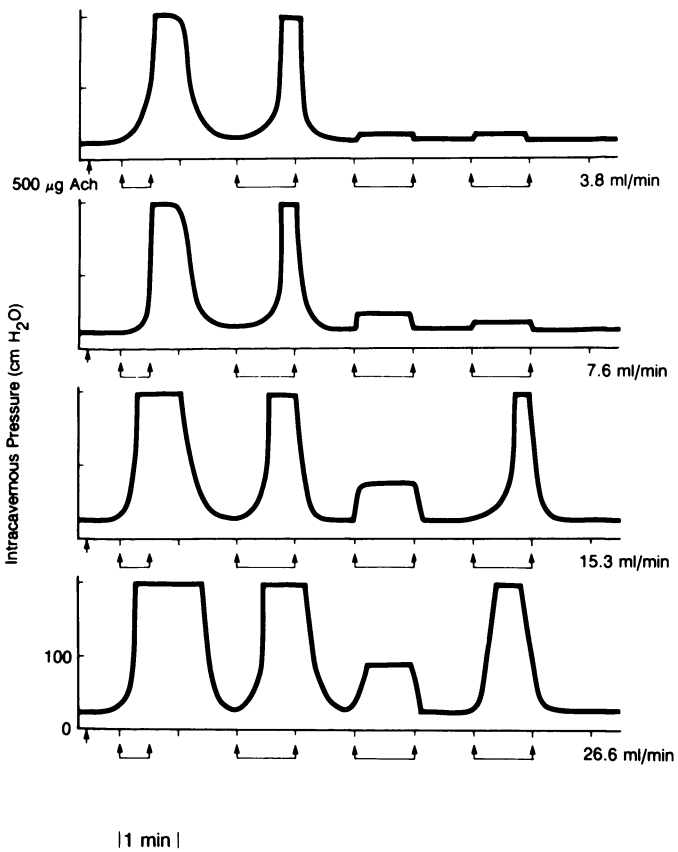

FIG. 3. With aorta clamped and 500 μg. ACh injected intracavernously, saline perfusion induced venous occlusion at minutes 0 and 1 (first tumescence phase). At minute 3, no venous occlusion could be induced by perfusion rates below 26.6 ml./min. (contraction phase). Then, at minutes 5 and 7, venous occlusion was produced by flow rates of 15.3 ml./min. and greater.

DISCUSSION

Intracavernous injection of ACh in the monkey induced a triphasic, dose-dependent erectile response: a first phase of penile tumescence and rigidity; a phase of penile contraction; and a second phase of penile tumescence, not as pronounced as the first. This triphasic reaction with contraction between two phases of tumescence may be explained by an immediate or reflexogenic release of noradrenaline from noradrenergic nerves.[20] Carbachol has been reported to induce a similar effect in vitro: cavernous smooth muscle relaxation followed by contraction.[15] After the effect of noradrenaline on its receptors has subsided, the second phase of tumescence could be explained by the ongoing effect of ACh on the cavernous smooth muscles in combination with the ongoing increase in arterial flow. The initial small dip in the intracavernous pressure at the beginning of this second phase of tumescence (fig. 2) can easily be explained by the elongation of the penis at this time.

Intracavernous ACh provoked an immediate, strong increase in arterial flow, which subsided somewhat but remained at moderately increased levels during the three phases. These pressure and flow effects were not due solely to a muscarinic cholinergic effect because they could not be completely blocked by atropine. Only after the additional intracavernous injection of a nicotinic blocker did ACh induce no erectile response. These observations correspond to the findings of Adaikan and

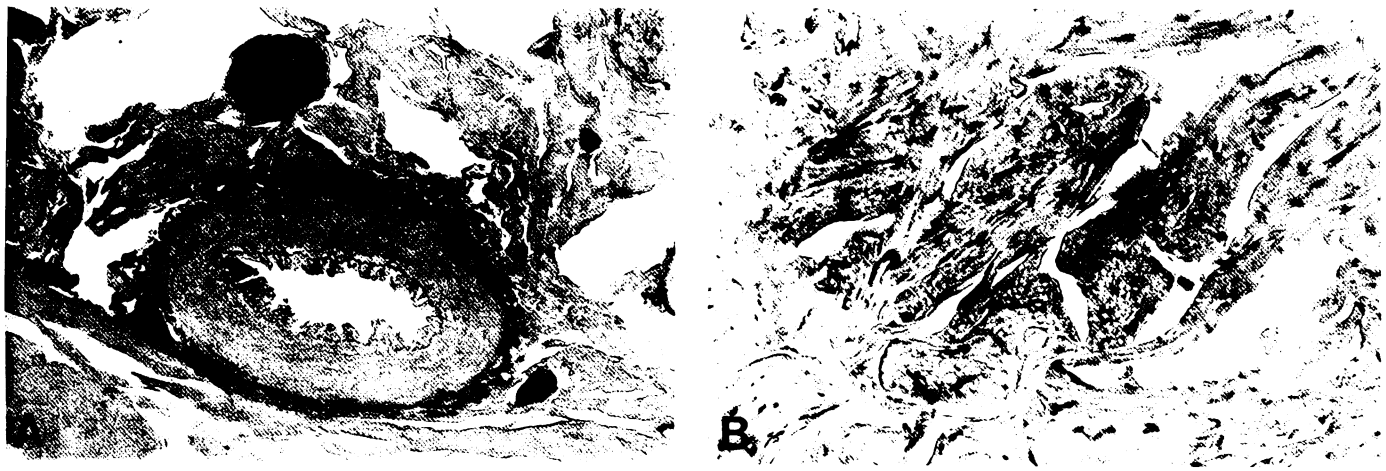

FIG. 4. Dense acetylcholinesterase-positive areas (dark spots) were found *A*, around cavernous arteries and *B*, within cavernous erectile tissue. (Magnification × 200 and × 400 for *A* and *B*, respectively.)

coworkers,[13] who demonstrated muscarinic and nicotinic cholinoreceptors in human cavernous tissue. This overlapping of muscarinic and nicotinic effects after the intracavernous injection of ACh, together with an increasing effect on the systemic blood pressure with doses of 0.1 and 0.5 mg., can explain the two (or more) wave-shaped erectile responses to high doses of ACh in seven monkeys.

Atropine modulated but did not abolish the erectile response to neurostimulation. The reduction of the immediate increase in arterial flow led to a prolongation of the tumescence phase. This and the decreased intracavernous pressure were supported as signs of incomplete cavernous relaxation by the staining of AChE around the cavernous artery and within the cavernous smooth muscles on histologic examination.

Our findings of the erectile response to intracavernous injection of ACh are in agreement with other in vivo studies: partial or full erections have been described after ACh in the rabbit[21] and dog;[22] atropine has been shown to reduce significantly the erectile response to pelvic and hypogastric nerve stimulation in the dog,[4] cat[23] and rabbit;[21] AChE-containing nerve fibers have been found in the cavernous tissue and around the cavernous artery in man and different animal species.[11,12,24-26]

At variance, Henderson et al. observed no erectile response after intraaortic injection of ACh.[3] This lack of effect may be due to the immediate inactivation of ACh by the AChE in the blood, to the pronounced lowering of the systemic blood pressure after high doses of ACh, or to the fact that the pressure in the glans instead of in the cavernous bodies was recorded. The ability of atropine to antagonize penile erection in man could not be proven in two studies.[9,10] However, intracavernous pressure was not monitored by invasive means and a 15 or 20% lowering of the maximal pressure could have been easily missed.

Results of in vitro studies of the effect of ACh on the cavernous smooth muscle have been contradictory, showing a range of results: no effect;[11,12] inconsistent effects;[13] contraction;[14] and relaxation.[15,16] These contradictory findings may have several causes. To prove a relaxing effect, the cavernous smooth muscle must first be contracted by electrical or pharmacologic means. In the studies in which the smooth muscles were contracted by norepinephrine before ACh was given, a relaxing effect could be shown. Secondly, the human cavernous smooth muscle, like the monkey's, will probably react to intracavernous injection of ACh in a triphasic manner, but the dog responds with a single phase of tumescence and rigidity (unpublished data). Thirdly, the action of ACh may be dependent on an intact cavernous endothelium (Goldstein, I.: personal communication). In the studies in which no effect was observed, the cavernous endothelium may have been damaged during preparation of the tissue.

Our study showed that intracavernous injection of ACh in the monkey induces a dose-dependent erectile response by increasing the arterial flow, relaxing the cavernous smooth muscles, and occluding the cavernous outflow. These in vivo results, together with the histologic findings of AChE-positive fibers around the cavernous artery and within the cavernous erectile tissue, suggest that ACh may be a neurotransmitter for penile erection. The fact that atropine could only diminish, but not abolish, the erectile response to neurostimulation suggests the presence of additional postganglionic parasympathetic neurotransmitters for erection. There is strong evidence that vasoactive intestinal polypeptide (VIP) may be one of these.[27-30] We have observed that the combination of atropine and anti-VIP likewise only reduces the erectile response to neurostimulation (unpublished data), and therefore further studies are needed to uncover additional neurotransmitters involved in penile erection.

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