

The Journal of
UROLOGY

FOUNDED IN 1917 BY HUGH HAMPTON YOUNG

Official Journal of the American Urological Association, Inc.

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VOLUME 143

BALTIMORE
WILLIAMS & WILKINS

1990

JOURAA

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A POSSIBLE ROLE FOR CALCITONIN-GENE-RELATED PEPTIDE IN THE REGULATION OF THE SMOOTH MUSCLE TONE OF THE BLADDER AND PENIS

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ABSTRACT

We investigated the effect of calcitonin-gene-related peptide (CGRP) on bladder contractions and penile erection in 12 dogs. In a system in which the arteries were tied bilaterally to ensure delivery of high drug levels to the bladder, arterial injections of CGRP significantly reduced the peak intravesical pressure of bladder contractions induced by pelvic nerve stimulation or arterial injection of carbachol. When given intravenously, CGRP had no effect on bladder contractions consequent to neural stimulation. Intravesical instillation of CGRP, however, reduced the bladder contractions significantly. Histologic staining showed CGRP-immunoreactive nerve fibers within the smooth muscle layers of the bladder wall.

Intracavernous CGRP increased cavernous arterial flow and induced cavernous smooth muscle relaxation and venous outflow occlusion. Muscarinic blockade had no effect on the canine intracavernous pressure response to intracavernous injection of CGRP. Histologic staining for CGRP-immunoreactivity showed nerve-fiber-like staining within the cavernous arterial wall, the nerves running near the cavernous arteries, and the cavernous smooth muscles.

Our results suggest a possible role for CGRP in the regulation of the smooth muscle tone of the bladder and penis. (*J. Urol.*, 143: 392-397, 1990)

In 1983, Rosenfeld and coworkers reported that the RNA that codes mRNA for calcitonin in thyroid C cells codes an alternative mRNA in neural tissue.¹ The latter mRNA codes for a 37-amino-acid peptide, calcitonin-gene-related peptide (CGRP), whose distribution is distinctly different from that of calcitonin. CGRP immunoreactivity has been found extensively throughout the central¹⁻³ and peripheral nervous system.⁴⁻²³ CGRP influences a variety of autonomic effects such as vascular tone,⁴⁻⁷ smooth muscle contraction⁸ and relaxation,⁹⁻¹⁵ and striated muscle relaxation.¹⁶ Additionally, recent findings of CGRP-I in sensory ganglia and nerve fibers, as well as in the adrenal medulla and pituitary gland,^{17,18} suggest that CGRP may have sensory and endocrine functions.

High concentrations of CGRP-I have been reported within the urinary bladder (and the regions of the spinal cord essential for bladder regulation) and the penis.¹⁹⁻²² Thus, in addition to CGRP's sensory functions, modulating effects on the vesical smooth muscle have been assumed.^{21,22}

Regarding penile erection, many studies have been undertaken recently to examine potential neurotransmitters.²⁴⁻³¹ Since acetylcholine, the presumed postganglionic parasympathetic neurotransmitter,³² was shown not to be the only neurotransmitter for erection,^{33,34} other substances have been investigated. The aim of our study was to examine in vivo the effects of CGRP on the canine urinary bladder and the cavernous bodies.

MATERIALS AND METHODS

In 12 adult male mongrel dogs (24 to 39.5 kg.), anesthesia was induced by acepromazine (five mg./kg. BW) and ketamine (0.5 mg./kg. BW) subcutaneously. Sodium pentobarbital (approximately one mg./kg. BW/hr intravenously) was used to maintain anesthesia. Systemic arterial blood pressure was monitored via a cannula in the axillary or femoral artery for the

bladder and erection studies, respectively. Anticoagulation was assured by bolus injection of 1000 U sodium heparin intravenously and was maintained by infusion at 50 U/hr.

Bladder study (six dogs). With the animal in the supine position, the abdominal cavity was exposed via a mid-line incision. The pelvic nerve was identified by neurostimulation with a needle electrode (Avery Labs). It was dissected and a cuff electrode (Avery) was placed distal to the branching of the cavernous nerve and proximal to the point where the hypogastric nerve joins the pelvic nerve (fig. 1). Stimulation was done with an Avery stimulator (stimulation parameters: 2.5 V, 20 Hz, 1-msec square waves, 20-sec duration).

A 22-gauge catheter (Intracath; Desert Medical Inc.; Sandy, Utah) was inserted into the left external iliac artery and its tip placed proximal to the aortic trifurcation. The following arteries were tied to ensure high CGRP levels in the bladder: the external iliac, the parietal branch of the internal iliac, the pudendal and lumbar (L₅, L₆) arteries bilaterally; and the sacral artery. Intravesical pressure was measured with a 5-gauge catheter (Argyle Suction Catheter; Sherwood; St. Louis, MO) and monitored on a Grass Polygraph (Mod.7) with the aid of Satham transducers (Mod. P 23 BC).

Arterial injection of CGRP. The pelvic nerve was stimulated at three-minute intervals. If voiding occurred, the volume was replaced with saline at 37C. The bladder was filled to about half capacity (mean 58 ml.). After four control stimulations, one $\mu\text{g.}$ (0.26×10^{-9} mol) human CGRP (Sigma Chemicals, St. Louis, MO) was injected one min. before the next stimulation in all six dogs. Further pelvic nerve stimulations were done until bladder contractions equal to the control contractions were elicited three times.

In dogs 4 to 6, 10 ng carbachol (Alcon Inc., Puerto Rico) was injected intraarterially three times with an interval of seven minutes between injections. CGRP (one $\mu\text{g.}$) was then injected intraarterially and 10 ng carbachol was again given after two min. Carbachol injections were repeated until bladder contractions equal to those before CGRP were elicited twice.

Accepted for publication September 14, 1989.

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Supported by a grant from the Deutsche Forschungsgemeinschaft.

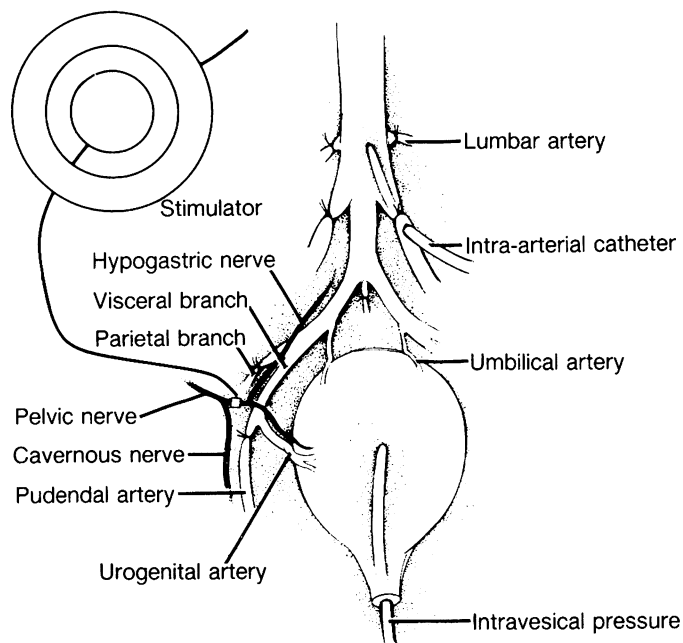


FIG. 1. Schematic illustration of experimental set-up for bladder study (see Methods).

Intravenous injection of CGRP. The same stimulation protocol was performed in two dogs (1 and 2) with intravenous injection of 250 ng./kg. BW.

Intravesical instillation of CGRP. After three control stimulations, CGRP (500 mg./kg. BW) was instilled through the catheter in dogs 1 to 3. After another six stimulations, the bladder content was replaced by the same amount of normal saline at 37°C. Five minutes later, neurostimulations were repeated until three bladder contractions equal to the control contractions were elicited.

Erection study (six dogs). With the animal in the supine position, the abdominal cavity was exposed via a midline incision. An ultrasonic flow probe (Transonic Systems Inc.; NY) was placed around the right internal pudendal artery. The cavernous nerves were identified by neurostimulation with an Avery needle electrode distal to the branching of the pelvic nerve. A bipolar cuff electrode (Avery Labs) was placed around the cavernous nerve bilaterally and erection was induced with an Avery stimulator (0.6 to 1.2 V, 20 Hz, pulse duration one msec, stimulation time one min).

The penis was dissected free in all dogs, exposing both corpora cavernosa from the pubic bone to the prepuce. For cavernous pressure recording (Grass Polygraph Mod. 7) (fig. 2), a 21-gauge scalp-vein needle was inserted proximally into each cavernous body and connected to a Statham transducer (Mod. P23). To evaluate the cavernous drainage system without the influence of arterial flow, the aorta was dissected for temporary occlusion with a Satinsky clamp proximal to the aortic trifurcation. For continuous cavernous perfusion, a 19-gauge scalp-vein needle was inserted distally into each cavernous body and connected to a Harvard perfusion pump.

Intracavernous injection of CGRP. In all dogs, 500 ng CGRP (0.13×10^{-9} mol) was injected into the right cavernous body, and one hr. later, the same amount was injected into the left cavernous body (the dog has a complete septum).

The effect of CGRP on the cavernous outflow. Thirty seconds after clamping the aorta, the left cavernous body was perfused with saline solution (37°C) at a rate of 7.6 ml./min. before and after 500 ng CGRP intracavernously. Perfusions were performed every five min. for one min or until the intracavernous pressure rose off the scale. The perfusion protocol was stopped when two consecutive perfusions induced an intracavernous pressure response equal to the control.

Erections induced by neurostimulation before and after CGRP-antibody. Erections were induced by neurostimulation three times before intracavernous injection of 500 ng CGRP antibody (Peninsula Lab.; San Carlos, CA). Thereafter, stimulations were repeated until three consecutive erectile responses equal to the control responses were elicited. The interval between each stimulation was five min.

Intracavernous injection of CGRP after muscarinic blockade. To examine the erectile effect of CGRP after muscarinic blockade, 500 ng CGRP was injected intracavernously five min. after intracavernous injection of 0.1 mg. atropine (1.4×10^{-7} mol). In a previous study in dogs, 0.1 mg. atropine intracavernously proved to be sufficient to abolish the erectile response to intracavernous injection of acetylcholine (data not shown).

Histological staining for CGRP immunoreactivity. In two other dogs that were to be sacrificed and in which no bladder studies had been done, the arteries were tied as above. After clamping the aorta above the level of the trifurcation, a 16-gauge cannula was inserted distally and 1000 ml. phosphate buffer (0.1 M) and 1000 ml. phosphate-buffered paraformaldehyde (4 percent) were infused. All solutions were at 4°C. One square centimeter of tissue was cut from the bladder dome and the trigone and placed in the fixation solution for 30 min. at 4°C.

In two more dogs to be sacrificed, the aorta was clamped and the cavernous bodies were perfused with 500 ml. saline, followed by 500 ml. phosphate buffer (0.1 M), and fixed with 500 ml. phosphate-buffered paraformaldehyde (4 per cent). The cavernous bodies were removed and sections approximately 0.5 cm. long were placed in the fixation solution for 30 min at 4°C. After overnight rinsing in 30 per cent sucrose, the tissues were embedded (Tissue Tek; Miles Scientific; Naperville, IL) and frozen.

An indirect immunofluorescence technique was used to locate CGRP-immunoreactive fibers.³⁵ Cryostat sections (16 μ m.) were cut and placed on lycine-coated slides. The sections were rehydrated with buffer (0.05M PBS + 0.3% Triton-X 100, pH 7.4) for five min., then preincubated at room temperature for 30 min. with five per cent goat serum in buffer. After draining but not rinsing, the sections were incubated in a humidified chamber for 48 to 72 hr. at 4°C with an antibody to CGRP raised in rabbits (Peninsula Lab.; San Carlos, CA), dilution 1:100. After three 10-min. washes in buffer, the sections were incubated for 30 min. at 37°C in goat anti-rabbit IgG conjugated to fluorescein-isothiocyanate (FITC) (Cooper Biomedical; Malvern, PA) diluted 1:50. The sections were then washed, mounted with glycerol:PBS (9:1) containing 1.5 per cent 1,4-diazobicyclo [2.2.2] octane, pH 8.6 (DABCO, Sigma Chemical) and viewed with an Olympus microscope equipped for viewing FITC fluorescence. As a control, tissues were prepared as above but without CGRP-antibody.

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

RESULTS

Bladder study: arterial injection of CGRP. Unilateral pelvic nerve stimulation induced bladder contractions with an increase in intravesical pressure of 30 to 52 cm. H₂O (mean 40.7) above baseline. Arterial injection of one μ g. CGRP lowered the systemic blood pressure from a mean of 180/150 to 150/120 cm. H₂O for about three min. The first nerve stimulation after CGRP elicited a slightly reduced peak intravesical pressure. The following four stimulations (four to 13 min post-injection) resulted in significantly reduced peak pressures. However, the contractions at minute 17 and later were similar to the control (fig. 3, table 1).

Arterial injection of carbachol was followed, after about six seconds, by a lengthy bladder contraction (210 to 300 sec) with a peak pressure of 22 to 37 cm. H₂O (mean 28) above baseline. Furthermore, carbachol decreased the systemic blood pressure by 30 cm. H₂O for two to three min. Two minutes after intraarterial CGRP, the peak pressure in response to carbachol

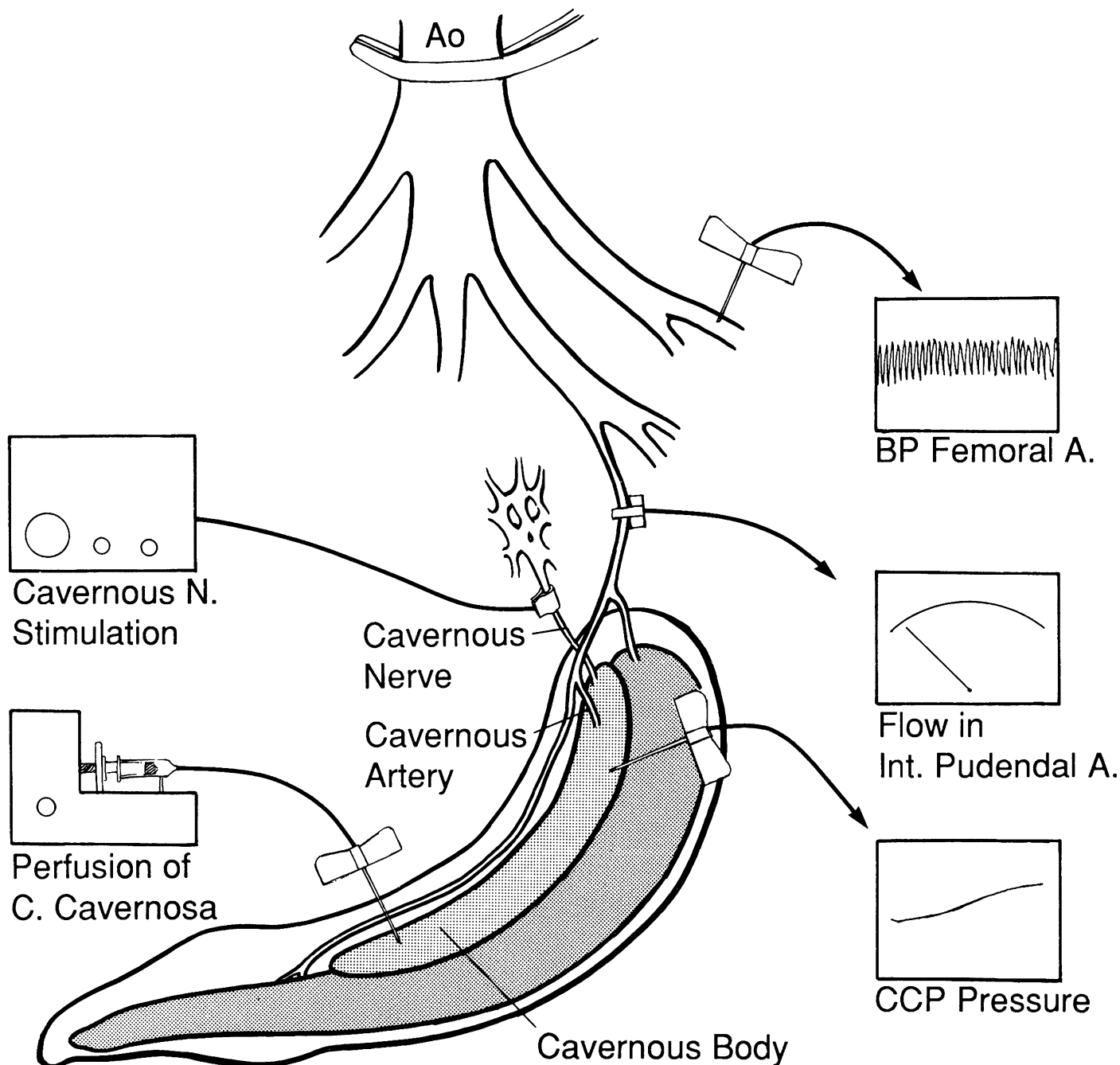


FIG. 2. Schematic illustration of experimental set-up for erection study (see Methods). CCP = pressure within corpus cavernosum.

injection was reduced to seven cm. H₂O above baseline in two dogs and was equal to the previous peak response in one. After another seven minutes, the carbachol-induced peak pressure was 11 (seven to 16) cm. H₂O above baseline. The duration of the bladder contractions after CGRP was similar to that before the injection. The bladder contractions from min. 16 onward were similar to the control contractions (fig. 4).

Intravenous injection of CGRP. Intravenous injection of CGRP was followed by a pronounced drop (60 cm. H₂O) in systemic blood pressure, which recovered slowly after one min. Neural stimulation-induced bladder contractions were unaffected.

Intravesical instillation of CGRP. Unilateral pelvic nerve stimulation induced a bladder contraction of 38 to 52 cm. H₂O (mean 46.1) above baseline. Intravesical instillation of CGRP provoked no changes in systemic blood pressure. With the next two stimulation-induced bladder contractions (four and seven

minutes post-instillation), the peak pressure rose off the scale (>60 cm. H₂O), but the subsequent stimulations induced a peak pressure of 25 to 35 cm. H₂O (mean 31) above baseline. Five minutes after replacing the bladder contents with saline, the peak pressures in response to the first neural stimulation were similar to the control peak, but the second stimulation induced a markedly higher peak pressure (off scale in two dogs).

Histologic staining for CGRP-immunoreactivity. In control slides and slides with CGRP-antibody, unspecific fluorescence of bladder endothelium and erythrocytes was found. In the slides with CGRP-antibody, isolated nerve-fiber-like and rare, but unequivocal, varicosity-like fluorescence was found within the smooth muscles of the bladder wall (fig. 5).

Erection study: intracavernous injection of CGRP. The baseline flow within the pudendal artery was 5.5 to eight ml./min. (mean seven). After the intracavernous injection of CGRP, the flow increased gradually over 120 to 600 sec (mean 345) to a

TABLE 1. Intravesical pressure increase due to bladder contractions induced by pelvic nerve stimulation

| Control | Minutes Post CGRP | | | | | |
|-------------|-------------------|-------------|-------------|--------------|-------------|-------------|
| | 1 | 4 | 7 | 10 | 13 | 16 |
| 40.7 ± 7.7* | 33.8 ± 9.4† | 23.8 ± 2.1‡ | 21.8 ± 4.3‡ | 25.8 ± 7.98‡ | 28.8 ± 5.2‡ | 35.4 ± 8.8† |

* Values are expressed as cm. H₂O, mean ± SD.

† p > 0.05 vis a vis control.

‡ p < 0.05 vis a vis control.

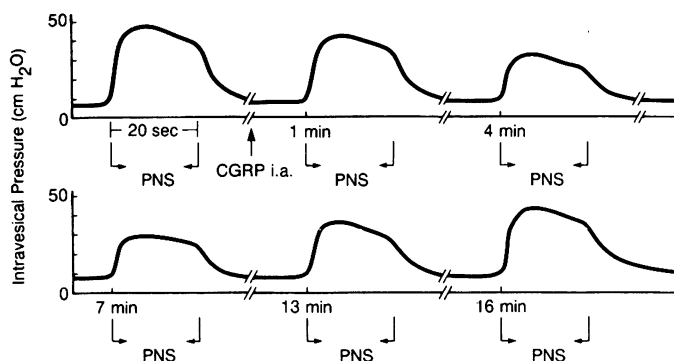


FIG. 3. Four to 13 seconds after intraarterial injection of CGRP, bladder contractions to neural stimulation were significantly reduced. PNS = pelvic nerve stimulation (stimulation time 20 sec).

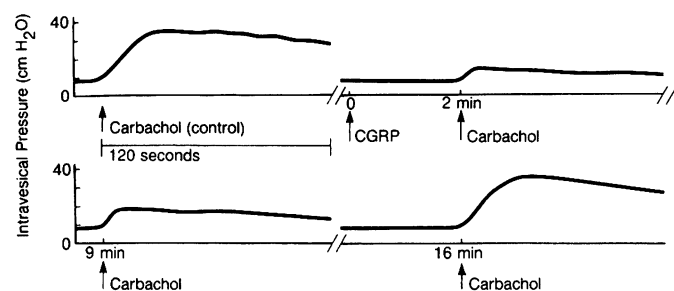


FIG. 4. Carbachol-induced bladder contractions were significantly reduced two and nine min. after intraarterial CGRP.

sec (mean 296). This pressure plateaued for a mean of 180 sec, then dropped within a minute to a mean of 42 cm. H₂O. The baseline intracavernous pressure was gradually reached 12 to 62 min. (mean 44) after intracavernous injection of CGRP. No changes in blood pressure or heart rate were observed.

The effect of CGRP on the cavernous outflow. After clamping the aorta, saline perfusion of the canine cavernous body at 7.6 ml./min. induced an intracavernous pressure that plateaued after about 15 sec at a mean of 51 cm. H₂O. One minute after 500 ng CGRP intracavernously, the pressure consequent to perfusion was equal to that before the injection. At repeat perfusion five minutes later, the pressure rose out of scale in all dogs (full venous occlusion due to cavernous smooth muscle relaxation). This venous occlusive effect was found for 45 to 105 min. (mean 65).

The intracavernous injection of 500 ng CGRP-antibody had no effect on the erectile response to neurostimulation, nor did muscarinic blockade (one mg. atropine) affect the erectile response to 500 ng CGRP.

Histologic staining for CGRP-immunoreactivity. In the control slides, nonspecific staining was found within the endothelium of the arteries. In the slides stained for CGRP-I, nerve fiber-like CGRP-I was found within the walls of the cavernous arteries, within the nerves running near the cavernous arteries, and within the cavernous smooth muscles. Additionally, we saw varicosity-like staining within the cavernous smooth muscles (fig. 6).

DISCUSSION

Our results show that, in this experimental system with the arteries tied bilaterally, arterial injection of CGRP reduced the neural stimulation-induced bladder contractions significantly. This seems, at least partly, to be due to a postganglionic effect because CGRP also significantly reduced the carbachol-induced bladder contractions. In addition, a ganglionic effect seems likely, since CGRP has been shown to prolong the duration of after-hyperpolarization of parasympathetic ganglia of the bladder.²³ Maggi and coworkers reported no effect of intraarterial CGRP on the rat bladder.¹⁴ However, this lack of effect may have been due to the injection site: CGRP was injected into the renal artery, which does not contribute to the arterial supply of the bladder. It therefore seems possible that the CGRP concentration was not sufficient to influence the smooth muscles of the bladder significantly.

In our study, intravenous injection of a dosage of CGRP that was about 10 times higher than that given intraarterially induced a dramatic decrease in systemic blood pressure, but failed to reduce the neural stimulation-induced bladder contractions.

Intravesical instillation proved to be effective in reducing bladder contractions. Shortly after CGRP was instilled, an increased bladder contraction to neural stimulation was observed, possibly owing to a reflexogenic increase in contractility induced by the abundant CGRP-containing sensory nerves of the bladder.¹⁹⁻²² This initial increase in contractility was followed by a significant reduction of the contractile response to stimulation, which was observed when CGRP was within the vesical cavity. As with the smooth muscle of the vas deferens, in the bladder a postjunctional inhibitory effect of CGRP on excitation and contraction may be assumed.^{10,22} Replacement of the CGRP-enriched bladder contents by saline was followed by another brief increase in bladder contraction to stimulation.

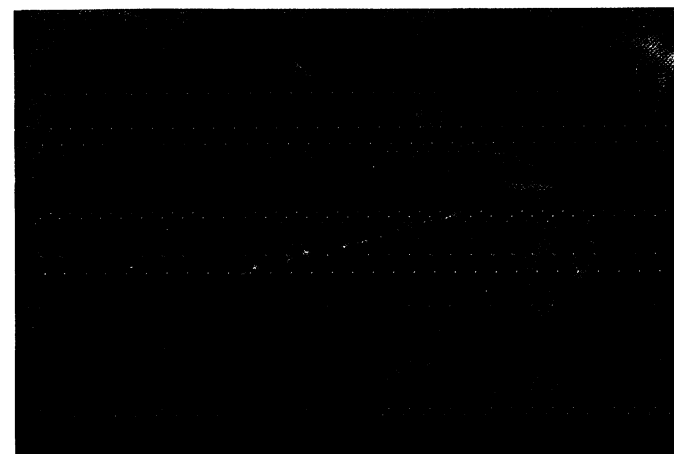


FIG. 5. Nerve fiber positive for CGRP immunoreactivity within smooth muscle cell of bladder wall (magnification × 125).

maximal rate of 7.5 to 16 ml./min. (mean 11.9). This maximal flow continued for eight to 26 min. (mean 15). After a slow decline, the baseline flow was reached after 23 to 50 min. (mean 41).

The baseline intracavernous pressure was 12 to 36 cm. H₂O (mean 20). After the intracavernous injection of 500 ng CGRP, the pressure began to rise gradually at 10 to 92 sec (mean 37) to a maximum of 48 to 132 cm. H₂O (mean 78) after 120 to 600

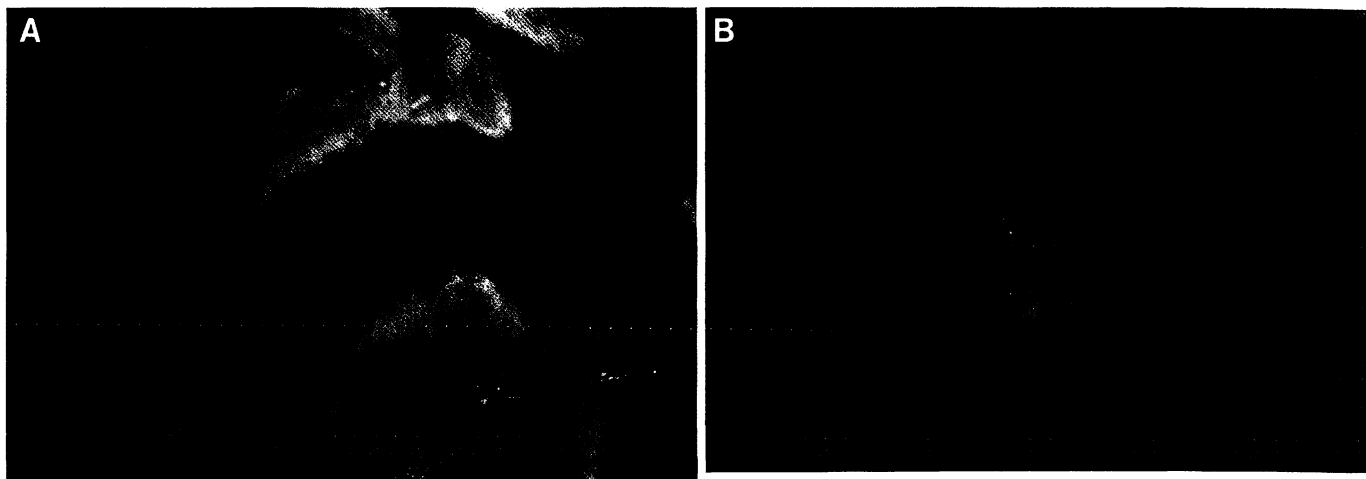


FIG. 6. CGRP-I was found within nerves near cavernous arteries (A) and cavernous smooth muscles (B) (magnification $\times 400$).

This terminal increase might also be due to CGRP-containing sensory fibers, in which the excitatory effect appears to be more sensitive than the inhibitory effect in the smooth muscle. This may be explained by the anatomical situation: the CGRP-containing sensory fibers are located below the endothelium of the bladder,¹⁹ and thus are reached immediately by intravesical instillation, whereas CGRP must penetrate the endothelium and subendothelial layer for smooth muscle relaxation.

Intracavernous injection of CGRP induced an erectile response by an increase in arterial flow, relaxation of the cavernous smooth muscles, and occlusion of venous drainage. The changes in arterial flow were shown on Doppler study of the distal pudendal artery. Relaxation of the cavernous smooth muscles by CGRP (and subsequent outflow occlusion) was demonstrated in the perfusion studies. The presence of CGRP-immunoreactive fibers in cavernous arteries and smooth muscles provides an anatomical basis for these *in vivo* findings.

Our histologic findings of CGRP-I in cavernous arteries and smooth muscles are supported by the results of Wimalawansa et al.,²² who found a high concentration of CGRP binding sites in rat penile tissue extracts. Arterial dilation in response to CGRP in man and different animal species has been found *in vivo*^{4,5} and *in vitro*.⁶ Our results showed the cavernous smooth muscle relaxing effect to be non-cholinergic. Similarly, CGRP has been found to induce striated muscle relaxation, acting on non-cholinergic receptors.¹⁶ Others have also demonstrated that CGRP-induced inhibition of the contractile response to electrical stimulation results from a direct effect on the smooth muscle.^{10,22}

Intracavernous injection of CGRP-antibody did not significantly affect the erectile response to neurostimulation. In contrast to VIP-antibody, which also has a VIP-antagonistic effect,³⁶ CGRP-antibody seems to have no CGRP-antagonistic effect.

Our findings support a possible role for CGRP as a neurotransmitter/cotransmitter for regulation of bladder smooth muscle tone and penile erection. Further studies are needed to investigate the possibility of therapeutic applications of CGRP in bladder hyperreflexia or related diseases, as well as in erectile dysfunction. An intrathecal or intravesical application seems promising for the treatment of bladder dysfunction. For the treatment of impotence, intracavernous injection may be tried, given the appropriate indications and careful patient selection.

Acknowledgment. We are indebted to Dr. A. Basbaum, Professor of Neuroanatomy and Physiology, for microscopy.

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