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Functional response of cavernosal tissue to distension

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Abstract We studied rabbit isolated erectile tissue responses to changes in preload and to active tension development with norepinephrine. The effects of antagonists of endothelin-1, prostaglandins E_2 and $F_{2\alpha}$ and of nitric oxide were also tested on normal and de-endothelialized preparations. Tissue distension was found to elicit spontaneous rhythmic contractions. Increase in preload diminished the latency of the spontaneous activity and augmented the developed force. Active tension development and the inhibitor of the Na^+,K^+ pump, ouabain, opposed the spontaneous activity. A marked reduction in the resting tension with abolition of the spontaneous activity was observed on normal, but not on de-endothelialized tissues, following the addition of the specific prostaglandin E_2 and $F_{2\alpha}$ receptor antagonist, SC-19220. At 3×10^{-4} M, the highest concentration used, the endothelin-A receptor antagonist BQ-123 failed to change the pattern of the spontaneous activity and the resting tension of normal tissues. The nitric oxide synthesis inhibitor, L-NAME, did not produce reliable effects. These findings point to a causal relation between cavernosal tissue distension and phasic and tonic contractions. Phasic contractions appear to be elicited by smooth muscle cells through the enzyme Na^+,K^+-ATPase. Increase in the resting tone could be mediated, at least in part, by the endothelium, through the release of prostaglandins E_2 and/or $F_{2\alpha}$ but not of endothelins. We discuss the hypothesis that, in cavernosal tissue, mechanotransduction of distension to contractile responses is an important determinant of detumescence.

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Introduction

Local modulation of adrenergic activity seems to be one of the most important means by which the contractile state of cavernosal smooth muscle and penile vessels is influenced [6, 17]. Withdrawal of adrenergic tone may be essential for the development of penile erection; a dominant non-adrenergic, non-cholinergic neurogenic influence induces the relaxation of trabecular and vascular smooth muscle [17]. In contrast, in the flaccid state and in the detumescence phase, these penile structures are contracted mainly by the release of norepinephrine (NE) from nerve terminals, but contributions of myogenic activity (owing to intrinsic smooth muscle tone) and of autacoids, i.e., prostanoids and endothelins, cannot be excluded, and are still not fully understood [3]. The aim of the present study was to characterize, at least in part, physiological mechanisms and agents possibly supporting the adrenergic component in the regulation of trabecular muscle tone.

It is well known that, when mounted in organ chambers and placed under isometric tension, isolated erectile tissues obtained from many species, including the human and the rabbit, display spontaneous contractile activity, which appears as rhythmic changes of tension (Fig. 1). This behavior is referred as myogenic activity [3]. Despite the fact that spontaneous contractile activity has been observed in penile tissues [2, 3], very little is known about its genesis. We asked whether spontaneous contractile activity could be imposed on the erectile tissue in response to distension. Thus, the effects on spontaneous activity of changes in preload and of active tension development, the latter induced with exogenous NE, were determined. Preliminary experiments disclosed that, apart from phasic effects (spontaneous contractions), distension of cavernosal tissue seems ca-

pable of modulating the resting tone via the endothelium. Hence, we found it of interest to monitor the resting tone, in normal as well in de-endothelialized tissues, in the presence of agents blocking endothelin (ET), prostaglandin (PG) and nitric oxide autacoids. Pharmacological tools we used included BQ-123, SC-19220 and N_ω-nitro-L-arginine (L-NAME). BQ-123 is a cyclic pentapeptide that inhibits binding of ET-1 to the ET_A receptor [13]. SC-19220 is reported to act as antagonist selective for prostaglandin E_2 and $F_{2\alpha}$ receptor [12]. L-NAME is widely utilized to inhibit nitric oxide synthesis from arginine.

Materials and methods

Preparation of erectile tissue

Erectile tissue was obtained from albino New Zealand male rabbits weighing approximately 3.5 kg. Animals were that had been killed and exanguinated underwent surgical excision of the entire penis. The penis was placed in chilled Krebs solution (for composition, see below) and immediately utilized. The corpora cavernosa were sharply dissected free from the tunica albuginea to produce erectile tissue strips $1 \times 2 \times 8$ mm. Care was taken throughout the procedure to minimize tissue manipulation. Strips were mounted in a 10-ml organ chamber with two metal holders, one of which was anchored and the other of which was connected to an isometric force transducer (Grass, model FT03). The transducer was connected to a positioner enabling tension adjustment (see below). Chambers contained Krebs solution (pH 7.4) of the following millimolar composition: sodium chloride, 118.3; potassium chloride, 4.7; magnesium sulfate, 0.6; monobasic potassium phosphate, 1.2; calcium chloride, 2.5; sodium bicarbonate, 25.0; and glucose, 11.1. The solution was maintained at 37 °C and continuously bubbled with 95% oxygen and 5% carbon dioxide. As a standard procedure, Krebs solution in the organ bath was not changed during the 90-min observation period, to avoid the dilution of endogenous substances possibly released from the tissue. Organ baths were throughly siliconized with dimethyldichlorosilane to avoid nonspecific adsorption of the peptides.

Tissue distension

The hypothesis of tissue distension as the stimulus for spontaneous contractions was investigated by utilizing normal strips set up with an initial tension (preload) of 5 ($n = 7$), 10 ($n = 7$) or 20 mN ($n = 6$).

Active tension development

For the experiments on active tension development tissues ($n = 5$) were almost unpreloaded, the tension being adjusted in the 0.0–0.5 mN range. Hence, NE (10^{-5} to 3×10^{-5} M) was added to attain a stable contraction of about 10 mN. Furthermore, two series of experiments were performed on strips preloaded at 10 mN showing spontaneous activity. In the first series of experiments strips ($n = 4$) developed active tension as a result of exogenous NE (3×10^{-6} M). Then, NE-induced contraction was counteracted with papaverine (10^{-6} M). In the second series of experiments preparations ($n = 4$) underwent electrical field stimulation in the presence of atropine (10^{-5} M). Electrostimulation consisted of a single 3-min train of supramaximal voltage (15 V) and 0.8-ms pulses at 10 Hz frequency. This was accomplished by means of two parallel platinum electrodes on either side of the strip (3 mm from tissue) connected to a current amplifier and stimulator (Grass, model S88) delivering single square-wave pulses. Active tension development following prolonged electrostimulation is consequent to the release of NE from intramural nerves [16].

Pharmacological assays

Pharmacological trials were carried out on tissues preloaded at 10 mN. Different assays were performed in separate experiments with cumulative addition of ouabain ($n = 4$), BQ-123 ($n = 6$), SC-19220 ($n = 5$) and L-NAME ($n = 9$). The effects of SC-19220 ($n = 5$) and L-NAME ($n = 9$) were also evaluated in de-endothelialized tissues (for the procedure of de-endothelialization, see below). Finally, separate experiments with SC-19920 were carried out in normal, almost unpreloaded preparations, where the addition of NE (10^{-5} to 3×10^{-5} M) was adjusted to reach a tension of approximately 10 mN.

Removal of the endothelium

To remove the endothelium, tissues were gently rubbed between the thumb and index finger for 25 s, according to Saenz de Tejada et al. [17]. Then, preparations were equilibrated at 10 mN. Possible damage of the smooth muscle component as a result of tissue manipulation was assessed by measuring the amplitude of tissue contraction to 3×10^{-6} M NE (see Results). In addition, successful removal of the endothelium was confirmed for each preparation by the inability of acetylcholine (10^{-5} to 10^{-3} M) to reduce the standard NE (3×10^{-6} M)-induced contraction.

Drugs and solutions

(±)-Norepinephrine, papaverine and acetylcholine chloride, cyclo(D-Asp,L-Pro,D-Val,L-Leu,D-Trp) (BQ-123), N_ω-nitro-L-arginine methyl ester hydrochloride (L-NAME), ouabain and dimethyldichlorosilane were purchased from Sigma (St. Louis, Mo.). 1-Acetyl-2-(8-chloro-10,11-dihydrodibenz(b,f)(1,4)oxazepine -10-carbonyl)-hydrazine (SC-19220) was a gift from Searle Chemicals (Chicago, Iu.). Stock solutions (0.25 M) of norepinephrine, acetylcholine, BQ-123 and L-NAME were prepared in distilled water. SC-19220 was dissolved in ethanol 10%. Papaverine and ouabain were dissolved in dimethylsulfoxide and ethanol, respectively. It was determined that dimethylsulfoxide and ethanol in the concentrations attained in the organ chamber during the experiments had no effects on the preparations. Aliquots of stock solutions of the above-mentioned drugs were frozen at −20°C, diluted each day and used within 60 days of preparation. Dilutions were obtained in distilled water. Concentrations in the experiments are reported as the final concentration in the organ chamber.

Calculations and statistics

To characterize the phenomenon of spontaneous contractions the following parameters were determined, within an observation period of 90 min: (i) latency, which constitutes the time between the end of the handling procedure and the appearance of the first contraction up to 0.5 mN; (ii) amplitude of the mean contraction, which is the mean of the isometric tension change developed by the single contraction; (iii) rate of contractions; (iv) "spontaneously developed tension", which was expressed as the product of the mean amplitude of contraction and the frequency.

Data are expressed as the mean ± standard error (SE). Statistical comparisons were performed by application of one-way analysis of variance, followed by the least-significant-difference test for comparison between groups, or by the unpaired Student's t-test for comparison of two groups. A chi-squared test was used to analyze the occurrence of spontaneous activity. Drug effects are expressed as the percentage of the basal tension (resting tone).

Results

Effects of change in preload and of active tension development on the spontaneous activity

Contractions up to 0.50 mN and to 1.5 events per minute were defined as spontaneous contractions. They were present in 80% of preloaded preparations. Data on tissue responsivity and on characterization of spontaneous contractions are reported in Tables 1 and 2. Spontaneous activity increased with increasing preload (Tables 1, 2); strips preloaded at 20 mN showed a significant decrease in the latency when compared with tissues preloaded at 5 and 10 mN, together with a significant increase in the parameter "spontaneously developed tension". None of tissues preloaded at 10 mN with NE (effective tension of 9.5 ± 0.8 mN) demonstrated oscillatory behavior (Fig. 1, Table 1). Exogenous NE and electrostimulation of preloaded tissues markedly reduced or abolished the oscillatory behavior (Fig. 2). Washout or papaverine reversed this effect, restoring in every case the spontaneous oscillations.

Table 1 Effects of preload and of active tension development on the genesis of spontaneous contractions (SCs). Based on a chi-squared test, the responsivity of preloaded tissues was found to be dependent on the magnitude of the tension applied, in a highly significant manner ($P < 0.001$)

Groups	Samples with SCs
5-mN preload	4 of 7
10-mN preload	6 of 7
20-mN preload	6 of 6
10-mN active tension development	0 of 5

Removal of the endothelium

It is noteworthy that the response of de-endothelialized strips to 3×10^{-6} M NE (8.26 ± 0.65 mN, $n = 20$) was similar when compared with that of non-de-endothelialized tissues (7.66 ± 0.69 mN, $n = 11$). This evidence fits well with no influence on the smooth muscle component following tissue manipulation. Acetylcholine (10^{-5} to 10^{-3} M) induced in every normal sample a marked relaxation (−80–100% of the standard NE-induced contraction), temporarily restoring the initial basal tone. This response was absent in all de-endothelialized strips which entered into the experimental protocol.

Table 2 Characterization of the activity of samples with spontaneous contractions (SCs). Mean ± SE. Statistics: one-way analysis of variance followed by the least-significant-difference test for multiple comparisons

Groups	Latency (min)	Amplitude of mean SC (mN)	Rate of SCs (contractions/min)	Spontaneously developed tension (mN/min)
5-mN preload ($n = 4$)	37.3 ± 12.3	1.83 ± 0.20	2.1 ± 0.8	3.84 ± 0.02
10-mN preload ($n = 6$)	38.8 ± 10.7	1.13 ± 0.28	7.9 ± 2.9	8.93 ± 3.65
20-mN preload ($n = 6$)	11.0 ± 3.2 *,†	2.21 ± 0.52	5.3 ± 1.2	11.71 ± 1.63 ***

* $P < 0.05$, *** $P < 0.001$ vs 5-mN preload
† $P < 0.05$ vs 10-mN preload

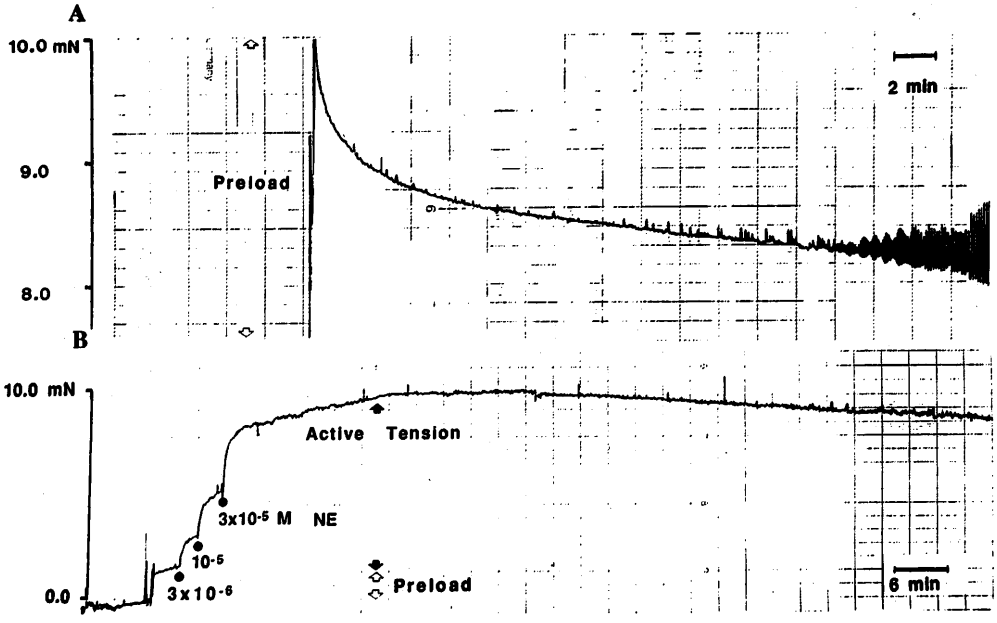

Fig. 1A, B Genesis of spontaneous rhythmic contractions. Effects of preload (**A**) and of active tension development (**B**). Tissues developing active contraction as a result of exposure to norepinephrine (*NE*) did not exhibit oscillatory behavior within the 90-min observation period

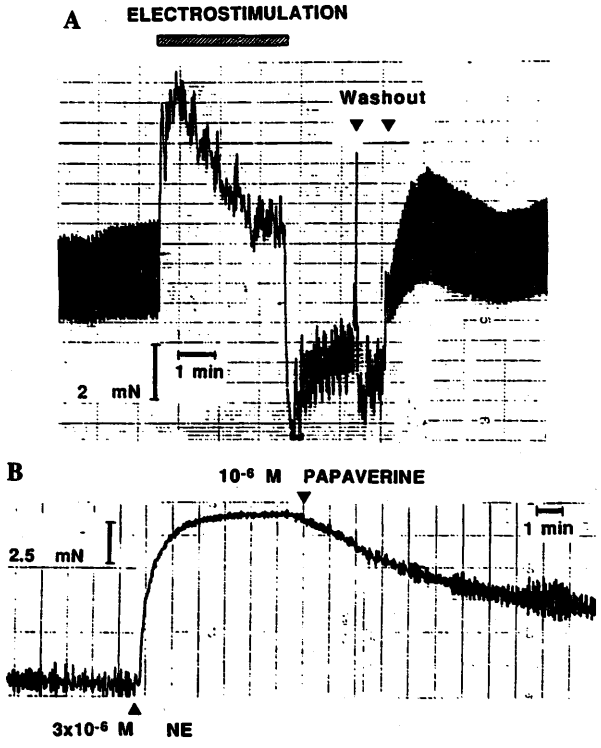

Fig. 2 Active tension development opposes spontaneous activity. **A** Electrostimulation produced a transient attenuation or cessation of spontaneous activity. When stimulation was stopped, spontaneous tension oscillations returned to their original pattern. **B** The addition of papaverine relaxed the tissue precontracted with norepinephrine (*NE*), and reversed the pattern of spontaneous contractions

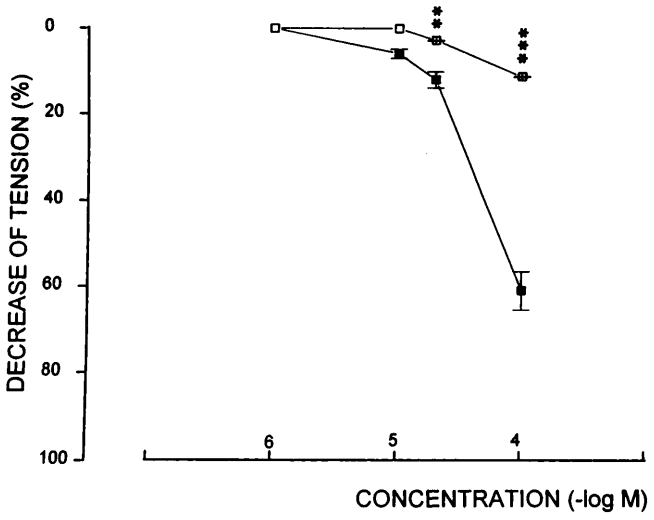

Fig. 3 Effect of SC-19220 on the resting tone of normal (*filled squares*, $n = 5$) and de-endothelialized preloaded strips (*open squares*, $n = 5$). Values are given as the mean ± SE. ** $P < 0.01$, *** $P < 0.001$. Student's *t*-test for unpaired data

Pharmacological assays

Inhibition of spontaneous activity was observed in all tissues ($n = 4$) treated with 3×10^{-6} M ouabain. BQ-123 (10^{-8} to 3×10^{-4} M) failed to reduce or enhance the tension of normal preloaded preparations. Conversely, SC-19220 induced a marked decrease in tension (Fig. 3): $-5.8 \pm 1.1\%$, $-11.9 \pm 1.9\%$, and $-60.8 \pm 4.5\%$ at 10^{-5}, 3×10^{-5} and 10^{-4} M, respectively. This effect was consistent with the abolition of spontaneous contractions. On de-endothelialized strips, SC-19220 produced a marginal decrease in tension of $2.7 \pm 0.1\%$ and $11.1 \pm 0.1\%$ at 3×10^{-5} and 10^{-4} M, respectively. When normal tissues were exposed to NE to develop active tension, SC-19220 did not show any effect.

Nine preloaded normal preparations were tested with the nitric oxide synthesis inhibitor, L-NAME (10^{-7} to 10^{-3} M). At a concentration of 10^{-5} M, L-NAME produced an abrupt augmentation of tension (+240–380%) in three specimens. One preparation responded at 10^{-4} M L-NAME (+73%). At a concentration of 10^{-3} M, L-NAME induced a slight increase in tension amounting to 28% and 31% in two preparations. L-NAME had no effect in three preparations. Together with the enhancement of tension, L-NAME induced a concomitant appearance of spontaneous contractions in two preparations. L-NAME up to 10^{-3} M had no effect at all resting tensions in nine of nine de-endothelialized strips.

Discussion

This study supports the following suggestions: (i) Distension of cavernosal tissue, when mounted in vitro, induces the phenomenon of phasic spontaneous contractions, in proportion to the degree of preload. (ii) Spontaneous activity is triggered by Na^+,K^+-ATPase. (iii) Apart from spontaneous tension oscillations, erectile tissue strips per se develop a tonic, contractile state when distended. This tone is maintained, at least partly, by the endothelium, via the release of PGE_2 and/or $PGF_{2\alpha}$ but not of endothelins. (iv) In terms of physiological implications, mechanotransduction of distension to contractile responses might be interpreted as a major factor by which detumescence is induced.

Present observations demonstrate that tissue distension might be the causal stimulus for evoking spontaneous rhythmic changes in tension. Thus, spontaneous activity was found to depend on the degree of the preload (Fig. 1, Tables 1, 2). It was not observed when unpreloaded tissues were exposed to NE. Furthermore, active tension development induced with exogenous NE as well as with electrostimulation opposed the spontaneous activity phenomenon; the latter was restored following papaverine-induced relaxation or washout. It may be argued that, opposing tissue distension, active tension development counteracts the phenomenon of spontaneous activity.

By showing that the antagonist of $PGE_2/PGF_{2\alpha}$ receptors, SC-19220, greatly reduced the resting tension, our experiments have disclosed that, apart from phasic responses such as spontaneous contractions, tissue dis-

tension per se develops a tonic contractile state. That is, the resting tension is the result of an intrinsic tissue tone, developed with distension, which superimposes on the resting load. The transduction of the stimulus of distension to functional responses such as spontaneous activity and the development of force could be mediated by the smooth muscle and/or the endothelial cells. Present data account for the involvement of both these components. Smooth muscle cells of large arteries have been found to possess an inherent ability to generate periodic spontaneous contractions, associated with fluctuations in voltage and intracellular Ca^{2+} concentration [5, 10, 21]. The abolition of spontaneous contractions with ouabain indicates that the cyclic mechanical activity of cavernosal smooth muscle is maintained by Na^+,K^+-ATPase. This enzyme is electrogenic and is known to affect the membrane potential of smooth muscle cells [7, 19]. Recent studies report that stretch stimulates the expression of α_1 and α_2 subunits of Na^+,K^+-ATPase in rat vascular smooth muscle cells [18].

As regards the endothelial component, present data suggest that it might mediate the transduction of the stimulus of tissue distension to tonic contraction. In fact, the resting tone of de-endothelialized tissues did not change following treatment with SC-19220. The absence of any change in tension of normal unpreloaded preparations contracted with NE, in the presence of SC-19220, further outlines the role of distension in stimulating the synthesis/release of local mediators. Precedents in literature report that indomethacin, an inhibitor of prostaglandin synthesis, lowered the resting tone of preloaded human erectile tissues, thus disclosing the presence of a stable cyclooxygenase product [1, 4]. Experiments with SC-19920 suggest that a basal release of PGE_2 and/or $PGF_{2\alpha}$ occurs from the endothelium with distension. The ET_A receptor antagonist BQ-123 did not affect the pattern of spontaneous contractions and the resting tone. Hence, it is unlikely that distension involves the production and/or release of ETs.

As regards nitroxide (NO), our experiments do not provide convincing evidence on the involvement of this mediator, and substantially confirm previous studies by Holmquist and colleagues [8]. These authors reported that inhibition of NO synthesis with L-NNA had little or no effect on isolated rabbit and human corpus cavernosum, when tested on preparations at basal tension. Conversely, a relaxant effect was observed in preparations where tension had previously been increased by NE. Thus, Holmquist et al. concluded that passive stretching of cavernosal tissue is not sufficient to induce continuous release of endothelium-derived NO, whose role might consist of the minute-by-minute control of cavernosal tissue tone in the flaccid state, when a sustained sympathetic input occurs. Holmquist's results were confirmed in other laboratories [9]. On this line, Vargas and coworkers (20) documented that the vascular tone may be physiologically regulated by a basal release of NO, and suggested that the physiological release of endothelium-derived NO is dependent on the tone of the vascular resistence in vivo.

Overall, our observations demonstrate that the cavernosal endothelium plays a critical role in the transduction of the stimulus of distension to contractile responses. Physiological studies indicate that several types of mechanical stress, including flow and pressure changes, lead to important biological changes in morphology and function of vascular endothelial cells, with stimulation of autacoid synthesis [11, 14, 15]. Endothelial cells seem to contain mechanotransducing Ca^{2+} channels, whose opening frequency increases with stretching of the cell membrane [10].

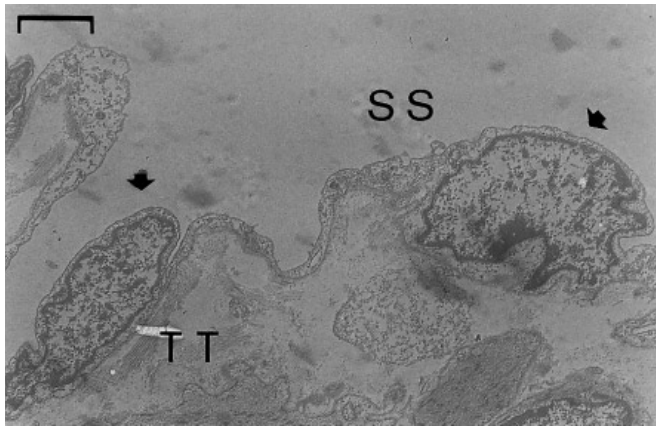

Fig. 4 Transmission electron micrograph of a cross-section of a cavernosal tissue specimen obtained from the flaccid penis. *Arrows* indicate the endothelial cell nuclei. *SS* sinusoidal space, *TT* trabecular tissue. The nucleus contains an inconspicuous nucleolus and fine chromatin particles. Broad endothelial cell nuclei characterize the flaccid cavernosal tissue. Scale bar represents 4.0 μm

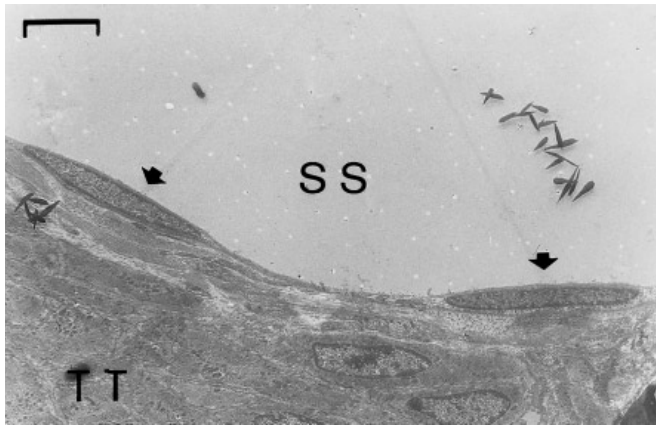

Fig. 5 Transmission electron micrograph of a cross-section of a cavernosal tissue specimen obtained from the erect penis. To obtain fixation of the erect penis an adequate volume of 10% formalin solution was injected into the corpora and erection was maintained 15 min before corpora dissection and standard processing of the samples. *Arrows* indicate the endothelial cell nuclei. *SS* sinusoidal space, *TT* trabecular tissue. Note the erection-induced overdistension of the body of the endothelial cell. Scale bar represents 2.8 μm

The physiological interpretation of the present results might be the contribution of mechanotransduction mechanisms to detumescence. Wall stress and accompanying tissue overdistension, imposed by the engorgement of blood within the lacunar spaces during erection (Fig. 4, 5), might elicit the increase in tone in the cavernosal smooth muscle; increased tone, in turn, reduces and abolishes pressure stress, which is the causal stimulus. Endothelial cells may possess the ability to transduce the mechanical stimulus to the underlying smooth muscle cells, through the synthesis and/or release of PGE_2 and $PGF_{2\alpha}$. In contrast, ET and NO release seem to be related to the maintenance and modulation of contraction in the flaccid state.

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