Erectile dysfunction in spontaneously hypertensive rats: pathophysiological mechanisms

DELPHINE BEHR-ROUSSEL,1 PHILIPPE CHAMIOT-CLERC,1 JACQUES BERNABÉ,1 KATELL MEVEL, LAURENT ALEXANDRE,1 MICHEL E. SAFAR,2 AND FRANÇOIS GIULIANO1,3

1Pelupharm, Domaine Centre National de Recherche Scientifique, 91190 Gif sur Yvette; 2Department of Internal Medicine, Broussais Hospital, 75014 Paris; and 3Groupe de Recherche en Urologie, Unité Propre de Recherche de l’Enseignement Supérieur, Medical University of Paris South, 94275 Le Kremlin Bicêtre Cedex, France

Submitted 13 July 2002; accepted in final form 4 November 2002

Bhvr-Oussel, Delphine, Philippe Chamiot-Clerc, Jacques Bernabé, Katell Mevel, Laurent Alexandre, Michel E. Safar, and François Giuliani. Erectile dysfunction in spontaneously hypertensive rats: pathophysiological mechanisms. Am J Physiol Regul Integr Comp Physiol 284: R682–R688, 2003. First published November 7, 2002; 10.1152/ajpregu.00349.2002.—Hypertensive men have a higher prevalence of erectile dysfunction (ED) than the general population. Experimental evidence of ED in hypertensive animals is scarce. This study evaluates the erectile function of spontaneously hypertensive rats (SHR) and age-matched normotensive Wistar-Kyoto rats (WKY) in vivo by the increase in intracavernosal pressure after electrical stimulation of the cavernous nerve (CN) and by isometric tension studies on corporal strips. Frequency-dependent erectile responses to CN stimulations were reduced in SHR. Phenylephrine induced lower corporal contractions in SHR although pD2 values were similar to WKY. Endothelium-dependent relaxations to ACh were impaired significantly in SHR, and indomethacin improved these relaxations in both WKY and SHR, the latter thus reaching values similar to WKY. Corporal relaxations to sodium nitroprusside were enhanced in SHR. Thus a dysfunctional α-adrenergic contraction of the corporal smooth muscle, an increased cyclooxygenase-dependent constrictor tone, and/or a defect in endothelium-dependent reactivity are associated with the altered erectile mechanisms in SHR. Drugs targeting endothelial dysfunction may delay the occurrence of ED as a complication of hypertension.

IN MEN WITH HYPERTENSION, prevalence of erectile dysfunction (ED) is significantly higher than in the general population (9, 11). In fact, 8–10% of untreated hypertensive patients are found to be suffering from ED once their hypertension is diagnosed (26). The question has been raised as to whether the higher rate of sexual dysfunction in hypertensive individuals is the result of hypertension per se and/or to antihypertensive medications. Strong arguments indicate a possible causative role for some antihypertensive drugs such as diuretics and noncardioselective β-blockers (4, 9, 17, 38). Nevertheless, the functional and structural modifications induced by hypertension may also be strongly implicated in ED even though pathophysiological studies of ED induced by hypertension are surprisingly scarce (6).

The basal tone of the corpus cavernosum (CC) smooth muscle is controlled both at the level of the central and peripheral nervous system (13, 15). The sympathetic nervous system principally ensures flaccidity by producing an α-adrenergic-dependent tone of the corporal smooth muscle maintaining the penis in a flaccid state and thus minimizing intracavernosal blood flow and pressure (ICP). Upon sexual stimulation, penile erection occurs in response to the activation of proerectile autonomic pathways and is greatly dependent on adequate inflow of blood to the erectile tissue. It requires coordinated arterial endothelium-dependent vasodilatation and sinusoidal endothelium-dependent corporal smooth muscle relaxation (2, 7). Nitric oxide (NO) is the principal peripheral proerectile neurotransmitter implicated, since it is released in response to a sexual stimulation both by nonadrenergic, noncholinergic (NANC) neurons and the sinusoidal endothelium to produce cGMP and relax corporal smooth muscle, resulting ultimately in increased ICP (3, 22). This increase in ICP activates pressure-dependent venoocclusive mechanisms to limit the outflow of blood, thus further promoting elevated ICP and erectile response. The increased blood flow is thus ultimately driven by the force of the arterial pressure (14). Therefore, any factors modifying the basal corporal tone in the flaccid state, the arterial inflow of blood to the corpora, and/or the synthesis/release of neurogenic and/or endothelial NO within the corpora may be involved in the pathophysiology of ED.

Several arguments support the concept that pathophysiological modifications induced by hypertension by itself may be implicated in the occurrence of ED.

Address for reprint requests and other correspondence: F. Giuliani, Dept. of Urology, CHU de Bicêtre, 78 rue du Général Leclerc, 94270 Le Kremlin Bicêtre Cedex, France (E-mail: giuliano@cyber-sante.org).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Among the hallmarks of hypertension are an increased peripheral sympathetic activity (20), an elevated vasoconstrictor tone, and decreased endothelium-dependent vasodilatations in arteries from both conductance and resistance vasculature (24, 25, 32). The resulting imbalance in regulatory vascular tone can either be linked to an increased release of vasoconstricting and/or reduced release of dilating substances. In fact, the cyclooxygenase pathway has been identified as mainly involved in vascular endothelium-dependent alterations observed in hypertension (25). Interestingly, an increased norepinephrine content has also been found in nonvascular tissue of spontaneously hypertensive rats (SHR), implying that hyperinnervation is not restricted to the vasculature and may include other sympathetically innervated tissue, i.e., the genitourinary organs (20, 28, 35). Furthermore, hypertension also induces modifications in the vascular structure, i.e., remodeling that could participate in an overall reduced dilatory capacity (12). Interestingly, remodeling may also occur at the corporal level (27, 34) and thus participate in the pathophysiology of ED by altering the mechanical properties of the erectile tissue.

The primary goal of this study was to demonstrate the occurrence of ED in a genetic model of hypertension, i.e., SHR. A reliable and standardized way to study erectile function in the anesthetized rat is to electrically stimulate the peripheral proerectile neural pathways and concomitantly measure the ICP and blood pressure (10, 13, 14, 30). To date, these experiments have not been reported in SHR. Our second objective was to investigate the pathophysiology of ED in SHR by studying the corporal endothelium-dependent and -independent reactivities in vitro, with particular attention paid to the cyclooxygenase pathway.

METHODS

Male 12-wk-old SHR, their age-matched normotensive Wistar-Kyoto rats (WKY), and age-matched Sprague-Dawley rats (SD) (Iffa-Credo, France) were housed 7 days before the experiments with free access to food and water. All procedures were performed in accordance with the Guiding Principles in the Care and Use of Animals established by the American Physiological Society (1) and the legislation on the use of laboratory animals (NIH publication no. 85–23, revised 1996, and Animal Care Regulations in force in France as of 1988).

Erectile responses were elicited by electrical stimulation of the cavernous nerve (CN) in anesthetized rats, as previously described (10, 13, 14, 30). Briefly, SHR (n = 5) and WKY (n = 5) were anesthetized with an intraperitoneal injection of xylazine (10 mg/kg) and ketamine (90 mg/kg), tracheotomized, and maintained at 37°C. In another set of experiments, WKY, SHR, and SD were anesthetized with an intraperitoneal injection of urethane (1.2 g/kg). The carotid artery was catheterized to record arterial pressure, and a 21-gauge needle was inserted in one of the CC of the penis to record ICP simultaneously via pressure transducers (Elektronik 750). The CN was exposed at the lateral aspect of the prostate and mounted on a bipolar platinum electrode connected to an electrical stimulator (AMS 2100). For each animal, electrical stimulations of the CN (square-wave pulses of 1 ms, duration of 45 s, 6 V) at different frequencies (1, 2, 3, 4, 5, and 10 Hz) were performed in a randomized manner and repeated two times in view of establishing frequency-response curves. The erectile responses elicited by each electrical stimulation were quantified by calculating the ratio ΔICP (mmHg)/MAP (mmHg) × 100, with ΔICP being the difference between ICP in the flaccid state, i.e., before stimulation and ICP during the plateau phase and with MAP being the mean arterial pressure during the plateau phase. This ratio accounts for the strong influence of the systemic blood pressure in the amplitude of ICP increase during the plateau phase (14). At the end of the experiments, rats were killed with an overdose of urethane.

In vitro experiments, SHR (n = 12) and WKY (n = 12) rats were deeply anesthetized with pentobarbital sodium (50 mg/kg ip). In all rats, the penis was carefully harvested, transferred to ice-cold Krebs bicarbonate solution (in mmol/l: 118.0 NaCl, 4.6 KCl, 2.5 CaCl2, 1.2 KH2PO4, 1.2 MgSO4, 11.1 glucose, and 25.0 NaHCO3, pH 7.2–7.4), and dissected. Corporal strips (1–2 mm × 0.5 mm) were placed in 5-ml organ baths filled with Krebs maintained at 37°C, bubbled with 95% O2–5% CO2, and connected to a force-displacement transducer (Marty Technologie). Tissues were equilibrated for 45–60 min before being stretched progressively to their optimal tension defined in preliminary experiments (200 mg for both WKY and SHR). A concentration-response curve to phentolamine (Phe, 10⁻⁸ to 10⁻⁴ mol/l) was performed for each sample. Next, concentration-response curves to ACh (10⁻⁸ to 10⁻⁵ mol/l) were constructed on Phe-induced (10⁻⁵ mol/l or 3 × 10⁻⁵ mol/l, depending on the individual responses of each tissue to maintain comparable levels of stimulated forces between SHR and WKY) precontracted tissues in the absence and in the presence of indomethacin (10⁻⁵ mol/l). At last, responses of Phe-induced precontracted corporal strips to cumulative additions of sodium nitroprusside (SNP, 10⁻⁵ to 10⁻⁵ mol/l) were studied.

Values are expressed as means ± SE. For in vitro experiments, contractile responses are expressed as the absolute change in maximal developed tension (in g) normalized per gram tissue weight and relaxations as the percentage change in Phe-induced tone. Concentrations inducing 50% of the maximal effect were expressed as pD₂ values and calculated using GraphPad Prism (5). For both in vivo and in vitro experiments, comparisons were performed using two-way ANOVA with repeated measures followed by Bonferroni’s complementary analysis where relevant. A P value < 0.05 was considered to be significant.

RESULTS

Mean body weights of SHR were significantly lower than those of WKY rats (311.8 ± 3.9 vs. 395.2 ± 8.8 g, respectively, P < 0.005). Mean body weights of SD rats were significantly higher than those of WKY and SHR (445.8 ± 14.5 g, P < 0.001). MAP was significantly higher in anesthetized SHR compared with anesthetized WKY rats (169.9 ± 18.0 vs. 133.0 ± 5.7 mmHg, respectively, P < 0.01). Both MAP from WKY and SHR were higher compared with SD rats (15.7 ± 4.7 mmHg).

In vivo experiments. Typical erectile responses elicited by electrical stimulations (6 V, 10 Hz, 1 ms, 45 s) of the CN of WKY and SHR are displayed in Fig. 1. In both WKY and SHR, maximal electrical stimulation (6 V, 10 Hz, 1 ms, 45 s) of the CN induced a rapid increase in ICP associated with visible tumescence of the penis. This ICP increase was maintained as long as the stim-
ulation lasted (plateau phase) and, at the end of the 45-s period of stimulation, ICP returned directly to its prestimulation level. The profile of erectile responses was similar in SHR and in WKY (Fig. 1). The ICP/MAP ratio increased in parallel with the frequency of CN electrical stimulation both in SHR and WKY rats (Fig. 2A). However, the magnitude of the erectile responses was reduced drastically in SHR compared with WKY rats ($P < 0.01$), and this lower amplitude of erectile responses was found to be significant for all frequencies except 1 Hz ($P < 0.001$; Fig. 2A). When repeating these experiments in animals anesthetized with urethane, erectile responses in SHR are also reduced compared with WKY rats for all frequencies except 1 Hz ($P < 0.01$; Fig. 2A). Furthermore, we have compared these erectile responses with those obtained in normotensive SD rats and again observed reduced erectile responses in SHR compared with this control strain. Interestingly, each strain of control rats (SD and WKY) yields erectile responses of different magnitude, with those of WKY rats being slightly but significantly greater than those of SD rats at 3 and 4 Hz ($P < 0.05$; Fig. 2B).

In vitro experiments. Developed tensions to Phe ($10^{-8}$ to $10^{-4}$ mol/l) were significantly lower in corporal strips of SHR compared with WKY rats ($P < 0.001$, two-way ANOVA; Fig. 3), whereas the mean $pD_2$ value calculated for Phe was similar in SHR compared with WKY ($5.64 \pm 0.14$ vs. $5.87 \pm 0.05$; not significant (NS)).

During Phe-induced contractions (349.4 ± 34.3 vs. 399.1 ± 43.8 g/g wet wt, respectively, NS, Student’s $t$-test), endothelium-dependent relaxations to ACh were impaired significantly in SHR corporal strips compared with age-matched WKY rats (relaxations at $10^{-5}$ mol/l of 16 ± 7 vs. 31 ± 3%, $P < 0.05$, Fig. 4). Conversely, $pD_2$ values for ACh-induced relaxations were not significantly different between SHR and WKY rats.

In the presence of indomethacin ($10^{-5}$ mol/l), the ACh-induced relaxations were improved significantly in the CC of both WKY (31 ± 3 vs. 43 ± 4%) and SHR [16 ± 7 vs. 38 ± 8%, the latter achieving a relaxation similar to that in the WKY tissues (NS, 2-way ANOVA with Bonferroni’s complementary analysis); Fig. 4], whereas there were no loading tension differences between the two strains (359.9 ± 41.7 vs. 359.4 ± 34.5 g/g wet wt, respectively, NS, Student’s $t$-test).

Concentration-response relaxation curves were evoked by cumulative addition of SNP ($10^{-8}$ to $10^{-5}$ mol/l) on corporal strips precontracted with Phe ($10^{-5}$ mol/l) in the presence of indomethacin (Fig. 5). SNP-induced relaxations were enhanced significantly in corporal strips from SHR compared with WKY rats (maximal relaxations of 100 ± 1 vs. 74 ± 4%, respectively; $P < 0.005$), although stimulated forces attained with Phe in both strains were similar (254.8 ± 21.5 vs. 314.1 ± 37.1 g/g wet wt, respectively, NS, Student’s $t$-test).

**DISCUSSION**

In this study, we have determined that there are differences in the mechanisms involved in penile erection in vivo and in vitro in an experimental model of hypertension, the SHR. We have found that 1) the erectile responses induced by electrical stimulation of the CN in anesthetized SHR were reduced, 2) the endothelium-dependent relaxations of corporal strips were impaired in SHR, 3) cyclooxygenase products contributed to the normal and impaired endothelium-dependent relaxations in corporal strips of WKY and SHR, and 4) endothelium-independent relaxations...
were enhanced in corporal strips of SHR compared with WKY.

First, we have demonstrated that the magnitude of erectile responses was reduced considerably in SHR compared with age-matched WKY anesthetized with ketamine and xylazine. Interestingly, although this cocktail is the anesthesia of choice when working with these animals, we have repeated these experiments using urethane, which is known to have less effect on the cardiovascular system, at least in normal animals (16), and have reached the same conclusions concerning the reduction in erectile responses of SHR after electrical stimulation compared with WKY. We used 12-wk-old SHR, which are considered to be mature adults able to have normal reproductive behavior and which feature a fully developed increase of blood pressure and major alterations of both structural and mechanical vascular properties, with in particular an established decrease in the endothelium-dependent relaxations at all levels of the arterial tree (5, 12, 20, 24, 25, 32). Despite the fact that the magnitude of an erectile response is driven directly by the magnitude of the arterial blood pressure (14), we observed a severely reduced ICP plateau after electrical stimulation in SHR compared with normotensive rats from the same stock from which SHR are derived (WKY) or from a different strain (SD). A rise in ICP is an important predictor of penile rigidity and erectile function and, as such, is a major determinant of erectile capacity and function per se (14, 36). In normal rats, electrical stimulation of the CN elicits an increase in ICP within the same range of values as pressures necessary for

---

**Fig. 2.** Effect of cavernous nerve stimulation at increasing stimulation frequencies on the intracavernosal pressure (ICP) of SHR and WKY rats anesthetized with ketamine and xylazine (A) or Sprague-Dawley (SD), SHR, and WKY rats anesthetized with urethane (B). Results are expressed as the ratio $\frac{\Delta ICP (mmHg)}{MAP (mmHg)} \times 100$ during the plateau phase, with $\Delta ICP$ being the difference between ICP in the flaccid state and ICP during the plateau phase of the erectile response and MAP the mean arterial pressure during the tumescence phase. $**p < 0.001$, WKY vs. SHR, 2-way ANOVA. $**p < 0.01$ and $***p < 0.001$ vs. SD, 2-way ANOVA. $**p < 0.01$ and $***p < 0.001$, Bonferroni’s complementary analysis.
penile rigidity to occur in humans (60–90 mmHg; see Ref. 36). A decrease in the amplitude of the erectile response will lead to a decrease in penile rigidity, and thus ED. In this respect, it is likely that SHR is very predictive to assess the effects of hypertension on penile erection in hypertensive patients. Interestingly, SHR exhibit unchanged copulatory behavior but display fewer erections than normotensive rats (8). Very recently, other experimental models of hypertension have been used and describe decreased penile erections associated with hypertension as well (6, 18).

Hypertension is commonly associated with structural and functional modifications that take place at the level of the endothelium, vascular smooth muscle, and extracellular matrix of blood vessels (5, 12, 20, 24, 25, 32). Some studies have provided evidence that the penile vasculature could undergo similar modifications (18, 19, 27). However, the reactivity of the erectile tissue itself had never been studied to date. We found decreased developed tensions to Phe, an \( \alpha_1 \)-adrenoceptor agonist, in SHR corporal strips despite unchanged pD\(_2\) values. Interestingly, we have previously demonstrated that \( \alpha_1 \)-adrenoceptors are present and functionally relevant within the erectile tissue in rats (31, 37). The present results support the concept that there may be a modified responsiveness to \( \alpha \)-adrenergic stimulation in the corporal smooth muscle of SHR, which mediates, at least in part, some of the alterations in erectile mechanisms, as suggested previously (18), although the sensitivity of the contractile machinery of the smooth muscle to Phe is intact. A change in the expression of adrenoceptor subtypes in the corporal smooth muscle, similar to the one occurring in blood vessels of SHR (21), could explain the modified \( \alpha \)-adrenergic-mediated contraction of corporal strips from SHR.

In this study, we have also focused on the corporal endothelium-dependent and -independent relaxations implicated in the local physiology of penile erection. Interestingly, the Ach-induced relaxations were impaired significantly in corporal strips of SHR. Although endothelium-dependent impairment has already been well described in aortas and resistance arteries of SHR (23–25), impairment of endothelium-dependent responses in corporal strips of SHR has never been reported before. These observations are of major importance since endothelium-dependent relaxation of corporal smooth muscle plays a key role in the erectile response to sexual stimulation (7, 22). Interestingly, we found that cyclooxygenase-dependent products con-
tributed to the reduced endothelium-dependent reactivity of the erectile tissue in both SHR and WKY. Such results are in accordance with experiments establishing the role of vasoconstrictor prostanoids in rabbit corporal strips (3). Moreover, it has already been suggested that the release of endothelium-derived contracting factors such as cyclooxygenase products is augmented in hypertension (24), leading to an increase in the vascular constrictor tone (25, 32). Indeed, indomethacin improves the impaired ACh-induced relaxations in SHR to reach similar values of WKY. This leads us to propose that, in SHR, there is a higher vasoconstrictor tone resulting from an overproduction of cyclooxygenase products within the erectile tissue, making it harder for the corporal smooth muscle to relax and thus compromising erection. More importantly, these results indicate that there are similarities in the pathological functional modifications between systemic arterial and local corporal tissue reactivities in SHR, suggesting that the erectile tissue of SHR is not protected from the functional changes induced by hypertension. Moreover, previous experiments have suggested that structural changes exist in the penile vasculature from SHR (18, 19, 27), and functional alterations have also been described in rat resistance arteries (23). Indeed, these observations are also of interest, since the important control site for pressure is at the level of the small arteries/arterioles. However, because the concomitant release of other endothelium-derived substances than NO may also occur in these smaller vascular beds, the parallel with corporal smooth muscle relaxation mechanisms is more difficult to make since these substances have not yet been characterized in corporal smooth muscle. In any case, a decrease in both corporal smooth muscle relaxation and arterial relaxation, both participating in the reduction of blood inflow to the penis, are very likely to be, at least partly, responsible for the blunted erectile response observed in vivo.

Finally, we report an increase in endothelium-independent corporal relaxations elicited by the NO donor, SNP, in SHR compared with WKY. Thus the corporal smooth muscle of SHR retains its full ability to relax via the guanylate cyclase pathway. Even more, the sensitivity of the corporal smooth muscle from SHR to NO donors seems to be increased. This increased sensitivity or effectiveness could be a tissue-specific compensatory mechanism of the soluble guanylate cyclase pathway to defective endothelium-dependent relaxations (29). It is, however, not powerful enough to overcome the alterations induced by hypertension and responsible for the altered erectile responses to sexual stimulation, suggesting that other mechanisms are involved. In fact, in addition to NO from endothelial origin, NO released by NANC neurons at the level of the erectile tissue is also involved in the corporal relaxation. An altered release of neurogenic NO at the level of the erectile tissue could be another pathophysiological mechanism involved in hypertension-induced ED in SHR.

In summary, we report herein that ED occurs in a genetic model of hypertension and that there appears to be a common pathophysiological functional alteration between the reactivity of large and small vessels and the reactivity of the erectile tissue in genetically hypertensive rats. Indeed, we found a dysfunctional α-adrenergic-mediated contraction and blunted endothelium-dependent relaxations in SHR corporal strips. Conversely, we demonstrate an increase in corporal endothelium-independent relaxations in SHR. Based on these findings, we propose that the pathophysiology of ED in hypertension is the result of an increase in cyclooxygenase-dependent constrictor tone, although a defect of endothelial or neuronal NO production and/or bioavailability cannot be excluded. Additionally, remodeling resulting from hypertension may also significantly interfere with arterial blood inflow to the penis (34). The latter aspect needs further investigations. Considering these results, it is tempting to postulate that drug therapy improving vascular and corporal endothelial function (33) may be of interest to treat hypertension patients with ED.

We are grateful to Joël Ménard for precious scientific contribution to this work.

REFERENCES


27. Okabe H, Hale TM, Kumon H, Heaton JP, and Adams MA. The penis is not protected—in hypertension there are vascular changes in the penis which are similar to those in other vascular beds. Int J Impot Res 11: 133–140, 1999.


