

Impaired neurogenic and endothelium-dependent relaxant responses of corpus cavernosum smooth muscle from hyperthyroid rabbits

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Abstract

We investigated the effect of hyperthyroidism on the responsiveness of the rabbit corpus cavernosum smooth muscle. In male albino rabbits, hyperthyroidism was established by oral feeding of L-thyroxine at increasing dosages (150–450 µg/kg) over an 8-week period. This treatment produced a stable hyperthyroid state as indicated by the increased serum T₄ levels. The reactivity of corpus cavernosum tissue from hyperthyroid animals and euthyroid control animals was studied in organ chambers. Hyperthyroidism caused impaired neurogenic and endothelium-dependent relaxant responses with decreased E_{max} and pD_2 values. However, hyperthyroidism had no effect on both phenylephrine- and KCl-induced contractile responses and sodium nitroprusside- and papaverine-induced endothelium-independent relaxant responses, and there was no change in agonist potency. These data indicate that hyperthyroidism may impair both neurogenic and endothelium-dependent relaxation of corporal smooth muscle, and may contribute to the etiology of impotence. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Relaxation of cavernous smooth muscle is critical in inducing and maintaining penile erection. Relaxation of the corporal smooth muscle allows for the expansion of the lacunar spaces and compression of subtunical venules with entrapment of blood in the corpora during erection (Krane et al., 1989). Corpus cavernosum smooth muscle tone is controlled by both the nerves and the endothelium (Krane et al., 1989). It is well known that nitric oxide (NO) mediates both neurogenic and endothelium-dependent relaxation of the corporal smooth muscle (Ignarro et al., 1990; Rajfer et al., 1992; Azadzoi et al., 1992). Impairment of the mechanisms that support the relaxation of corpus cavernosum smooth muscle may lead to impotence (Saenz de Tejada et al., 1989; Pickard et al., 1994). Hyperthyroidism alters endothelium-mediated relaxation of blood vessels in experimental animals. There are many conflicting reports showing alterations in vascular responses in the hyperthyroid rat. For instance, Lockette et

al. (1987) reported that aorta obtained from hyperthyroid rats relaxed less to acetylcholine and generated significantly less cGMP in response to acetylcholine, while others have reported increased relaxation responses to acetylcholine (McAllister et al., 1998). Hyperthyroidism is a contributing cause of erectile dysfunction. Slag et al. (1983) found a 1% incidence of hyperthyroidism in a review of 188 impotent patients who presented to a general medical clinic. Thyroid hormone abnormalities may alter the hypothalamic–pituitary–gonadal axis, resulting in impotence. Hyperthyroidism causes both an increase in the estradiol production rate and a decrease in its metabolic clearance, resulting in elevation of serum estradiol level (Chopra and Tulchinsky, 1974; Kidd et al., 1979). Partial Leydig-cell failure is suggested in hyperthyroidism as basal serum luteinizing hormone (LH) levels are elevated and there is a blunted testosterone response to human chorionic gonadotropin (hCG). The Leydig cells may be inhibited by the high estradiol levels, by circulating antibodies to thyroid-stimulating hormone (TSH), or by both (Kidd et al., 1979). The decreased libido associated with hyperthyroidism may be due to the hypermetabolic effects of thyroxine or perhaps to the increased circulating estrogen levels

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(Kidd et al., 1979). Although it is reported that hyperthyroidism can cause erectile dysfunction, probably secondary to a reduced libido arising as a result of increased levels of circulating estrogen and sex hormone-binding globulin, the effect of thyroid hormones on the erectile response is unknown. In this background, the purpose of this study was to determine the effects of hyperthyroidism on the reactivity of penile corporal smooth muscle strips from euthyroid control and hyperthyroid animals.

2. Material and methods

Experiments were performed on mature male albino rabbits obtained from Experimental Medical Research Unit (DETAB, Kocaeli University, Kocaeli, Turkey) (2–2.5 kg). The albino rabbit was chosen as the model based on the close similarities that have been reported in the reactivity in vitro of human and rabbit corpus cavernosum.

2.1. Treatment schedule

Five rabbits were made hyperthyroid by daily oral administrations of increasing load of L-thyroxine (T_4 , suspended in water) according to the following schedule: 1st week 150 $\mu\text{g}/\text{kg}$ per day, 2nd week 225 μg , 3rd week 300 μg , 4th week 375 μg and 5–8th week 450 μg . Blood samples from treated and control animals were collected at the time of death and serum T_4 and TSH levels were measured by chemiluminescence methods (IMMULITE One automated analyzer).

2.2. Organ chamber experiments

Rabbits were anesthetized with ketamine and xylazine and exsanguinated as previously described (Utkan et al., 1999). Briefly, rabbit penises were surgically removed at the level of the crural attachments to the pubo-ischial bones, and the corpus spongiosum and urethra were excised. The corpus cavernosum tissue carefully dissected free from the surrounding tunica albuginea and strips of corpus cavernosum tissue were mounted in 20-ml organ chambers for isometric tension measurement. Each rabbit provided 3–4 strips of corpus cavernosum smooth muscle that were studied separately. The organ chambers contained Krebs–bicarbonate solution composed of (mM): NaCl 118, KCl 4.7, CaCl_2 2.5, NaHCO_3 25, MgSO_4 1.2, KH_2PO_4 1.2, glucose 11. The solution was gassed with 95% O_2 and 5% CO_2 during the study and the temperature was maintained at 37 °C by a thermoregulated water circuit. Each strip was connected to a force transducer (FDT 10A Commat Iletisim) for the measurement of isometric force, which was continuously displaced and recorded on an on-line computer via a four channel transducer data acquisition system (TDA 94, Commat Iletisim) using a software (Polywin 95 ver 1.0, Commat Iletisim)

that also had the capacity to analyse the data. After mounting, each strip was allowed to equilibrate with a basal tension of 2 g for 1 h and during this time Krebs–bicarbonate solution was replaced every 15 min with fresh solution. Corpus cavernosum tissue was isolated with intact endothelium as assessed by the capacity of acetylcholine (1 μM) to elicit relaxation.

At the end of the equilibration period, strips were depolarized with KCl 124 mM in Krebs–bicarbonate solution and allowed to equilibrate for 30 min. This procedure increases and stabilizes subsequent contractile responses to phenylephrine and decreases spontaneous contractile activity. After equilibration, the contractile responses to phenylephrine (10^{-9} – 10^{-4} M) were obtained cumulatively. After addition of each dose, we waited until a plateau response was obtained before adding the next one. Following completion of phenylephrine dose–response curve, tissues were washed for a further 30 min, precontracted with a submaximal concentration of phenylephrine (10^{-6} M). After the contraction reached a plateau, concentration–response relationships for acetylcholine (10^{-9} – 10^{-4} M), sodium nitroprusside (10^{-10} – 10^{-4} M) or papaverine (10^{-6} – 10^{-4} M) were obtained by adding one of those agents to the bath in a cumulative manner.

Electric stimulation was provided by a stimulator (ST95 PT, Commat Iletisim) and applied via two platinum wire electrodes set vertically within the opposite organ bath sides of the suspended tissue. Prior to electrical stimulation, tissue was treated with guanethidine (5 μM) (adrenergic nerve blocker) and atropine (1 μM) (muscarinic receptor blocker) for 30 min. Strips were precontracted with phenylephrine at 10^{-6} M; electric stimulation was then performed. Square-wave pulses of 10 V, 0.8-ms duration in 10-s trains with varying frequency (2, 4, 8, 16 and 32 Hz) were applied at 5-min intervals. The strips were allowed to return to baseline precontractile tension between the tests at each frequency. Two or three agonists were tested on each preparation.

2.3. Analysis of data

The results are expressed as the mean \pm S.E. of different experiments. Contractile responses to phenylephrine were calculated as percentage of the maximal contraction caused by KCl (124 mM). Relaxant effects of agonists

Table 1
General characteristics of hyperthyroid and euthyroid control rabbits

	Body weight (g)	T_4 (ng/dl)	TSH ($\mu\text{IU}/\text{ml}$)
Euthyroid control ($n = 5$)	2296.7 \pm 129.5	1.77 \pm 0.34	0.017 \pm 0.005
Hyperthyroid ($n = 5$)	2066.7 \pm 145.9	4.02 \pm 0.25 ^a	0.004 \pm 0.001 ^a

Values are arithmetic means \pm S.E., n = number of animals.

^a $P < 0.05$, statistically different from euthyroid control rabbits.

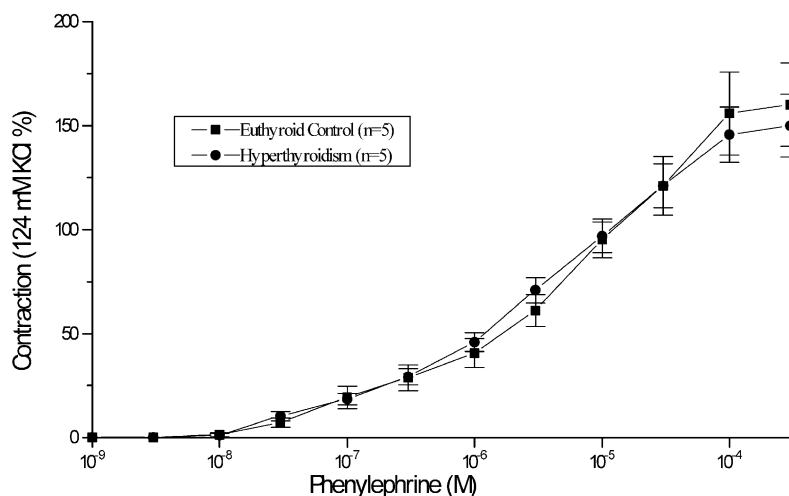


Fig. 1. Phenylephrine concentration–response curves in isolated rabbit corpus cavernosum strips. Each point is expressed as a percentage of the contraction induced by 124 mM KCl and is given as the mean \pm standard error of the mean (S.E.). Number of rats in each group is shown in parentheses.

were expressed as a percentage of the precontraction to phenylephrine. To evaluate the effects of agonists, pD_2 (i.e. the negative logarithm of the concentration for the half-maximal response; EC_{50}) and maximum response (E_{max}) values were calculated. Agonist pD_2 value was calculated from each agonist dose–response curve by linear portion of the curve and taken as a measure of the sensitivity of the tissues to each agonist.

Statistical comparison between groups was performed using Student's *t*-test. Results were considered to be significantly different at *P* value of < 0.05 .

2.4. Drugs

The following drugs were used: phenylephrine hydrochloride (Sigma), atropine sulfate (Sigma), guanethidine sulfate (Sigma), acetylcholine chloride (Sigma), L-thyroxine (Tefor, Organon), sodium nitroprusside (Nipruss, Adeca), papaverine hydrochloride (Sigma). All drugs were dissolved in distilled water and were freshly prepared on the day of the experiments.

Table 2

E_{max} (% of 10^{-6} M phenylephrine) values for acetylcholine, sodium nitroprusside and electrical field stimulation (EFS), E_{max} values (% of 124 mM KCl) for phenylephrine and E_{max} values (mg) for 124 mM KCl in corpus cavernosum strips obtained from hyperthyroid and euthyroid control rabbits

	Control	<i>n</i>	Hyperthyroid	<i>n</i>
Acetylcholine	89.12 \pm 10.43	5	46.23 \pm 6.89 ^a	5
EFS	64.9 \pm 10.9	5	32.3 \pm 5.40 ^a	5
Sodium nitroprusside	79.35 \pm 6.18	5	75.28 \pm 4.90	5
Phenylephrine	175.15 \pm 8.49	5	168.98 \pm 4.87	5
KCl	2094.83 \pm 494.28	5	2115.95 \pm 141.89	5

Values are arithmetic means \pm S.E., *n* = the number of rats.

^a*P* < 0.05 , statistically different from the response of strips from euthyroid control rabbits.

Ethical approval was granted by the Kocaeli University Ethics Committee (Kocaeli, Turkey, REC-7/74).

3. Results

The effect of T_4 -administration on animal weight and serum T_4 and TSH levels are shown in Table 1. Administration of T_4 was effective in inducing hyperthyroidism in rabbits. The initial body weight was similar in the control and hyperthyroid animals (data not shown). There was no significant difference in body weights of euthyroid and hyperthyroid rabbits after 8 weeks (Table 1).

3.1. Effects of hyperthyroidism on contractile responses

The cumulative addition of phenylephrine (10^{-9} – 10^{-4} M) produced concentration-dependent contractions of corporal strips. The contractility did not change in the strips from hyperthyroid rabbits. No change in pD_2 or E_{max} value for phenylephrine from hyperthyroid strips compared with euthyroid controls was detected (Fig. 1, Tables 2 and 3).

The contractions elicited by KCl (124 mM) were similar in two groups (Table 2).

Table 3

pD_2 values for phenylephrine, acetylcholine and sodium nitroprusside in corpus cavernosum strips obtained from hyperthyroid and euthyroid control rabbits

	Control	<i>n</i>	Hyperthyroid	<i>n</i>
Phenylephrine	5.12 \pm 0.19	5	5.25 \pm 0.08	5
Acetylcholine	5.56 \pm 0.18	5	6.50 \pm 0.28 ^a	5
Sodium nitroprusside	5.03 \pm 0.16	5	5.74 \pm 0.14	5

Values are arithmetic means \pm S.E., *n* = the number of rats.

^a*P* < 0.05 , statistically different from the response of strips from euthyroid control rabbits.

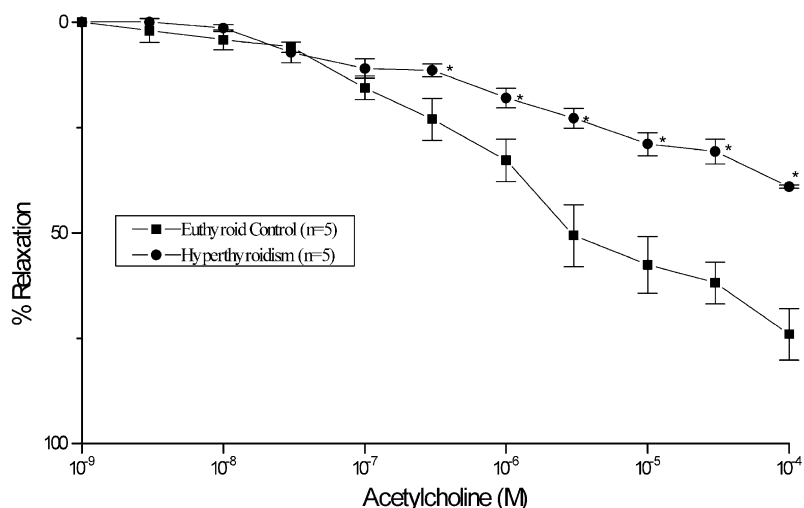


Fig. 2. Acetylcholine concentration–response curves in isolated rabbit corpus cavernosum strips precontracted with phenylephrine 10^{-6} M. Each point is expressed as a percentage of the contraction induced by phenylephrine and is given as the mean \pm standard error of the mean (S.E.). Number of rats in each group is shown in parentheses. * $P < 0.05$, statistically different from the response of strips from control rabbits.

3.2. Effects of hyperthyroidism on endothelium-dependent relaxation

Acetylcholine produced concentration-dependent relaxation in submaximally precontracted (10^{-6} M phenylephrine) corpus cavernosum strips obtained from euthyroid control and hyperthyroid rabbits. When tissues were contracted with phenylephrine for the study of responses to acetylcholine, similar tension was achieved in the hyperthyroid group and the control group. Tension induced by phenylephrine was 1677.8 ± 308 and 1697.4 ± 306.8 mg (mean \pm S.E.) in the hyperthyroid and euthyroid control groups, respectively. In the hyperthyroid state, the acetylcholine concentration–response curve was shifted to the right with significantly lower pD_2 values and E_{max} values

were inhibited compared with euthyroid control rabbits ($P < 0.05$) (Fig. 2, Tables 2 and 3).

3.3. Effect of hyperthyroidism on endothelium-independent relaxations

In precontracted strips, sodium nitroprusside produced concentration-dependent relaxations. When tissues were contracted with phenylephrine for the study of responses to sodium nitroprusside, similar tension was achieved in the hyperthyroid group and the control group. Tension induced by phenylephrine was 1897.6 ± 220.3 and 1842.7 ± 163.8 mg (mean \pm S.E.) in the hyperthyroid and euthyroid groups, respectively. The relaxation elicited by sodium nitroprusside was similar in hyperthyroid and euthyroid

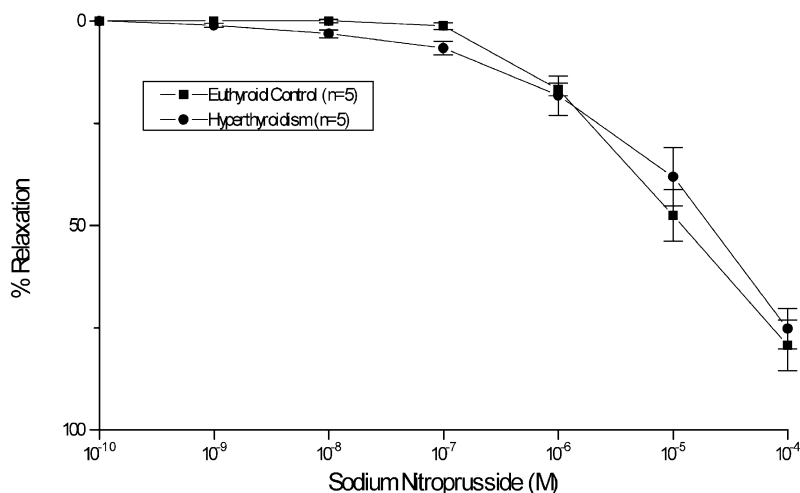


Fig. 3. Sodium nitroprusside concentration–response curves in isolated rabbit corpus cavernosum strips precontracted with phenylephrine 10^{-6} M. Each point is expressed as a percentage of the contraction induced by phenylephrine and is given as the mean \pm standard error of the mean (S.E.). Number of rats in each group is shown in parentheses.

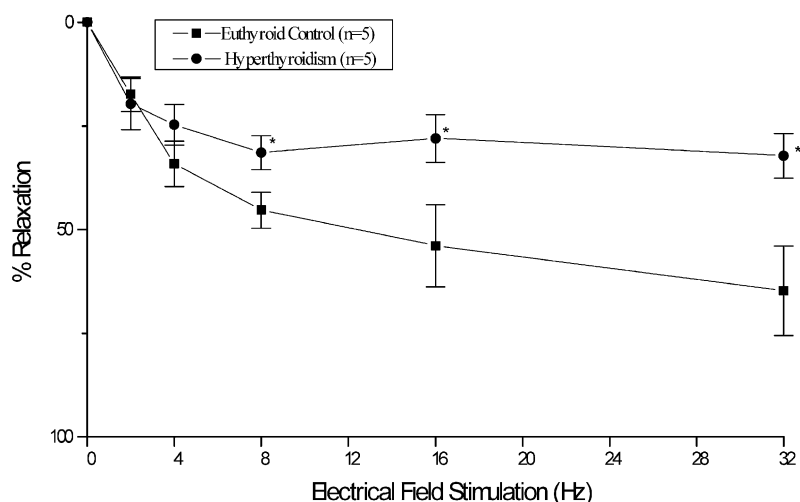


Fig. 4. The relaxation responses evoked by electrical field stimulation (EFS) of isolated rabbit corpus cavernosum strips precontracted with phenylephrine 10^{-6} M. Each point is expressed as a percentage of the contraction induced by phenylephrine and is given as the mean \pm standard error of the mean (S.E.). Number of rats in each group is shown in parentheses. * $P < 0.05$, statistically different from the response of strips from control rabbits.

control groups and there were no significant changes in the pD_2 or E_{max} values (Fig. 3, Tables 2 and 3).

The relaxant responses of corporal strips from hyperthyroid rabbits to papaverine (10^{-6} – 10^{-4} M) were not significantly different from those obtained with corporal strips from euthyroid rabbits (not shown).

3.4. Effects of hyperthyroidism on neurogenic relaxation

In precontracted strips, electric stimulation (2–32 Hz) evoked frequency-dependent relaxations. When tissue contraction was induced with phenylephrine for the study of responses to electrical stimulation, the amount of tone was similar in the hyperthyroid and euthyroid control group. Tension induced by phenylephrine was 1403.6 ± 389.8 and 1545.4 ± 280 mg (mean \pm S.E.) in the hyperthyroid and euthyroid control group, respectively. In the hyperthyroid group, electrical field stimulation responses were lower than those in the control group ($P < 0.05$) (Fig. 4, Table 2).

4. Discussion

The present study demonstrates that hyperthyroidism impairs neurogenic and endothelium-dependent relaxation to carbachol in the corporal smooth muscle while endothelium-independent relaxation to sodium nitroprusside or papaverine is fully preserved. Relaxation of cavernous smooth muscle plays an important role in penile erection. Studies of rabbit and human corporal smooth muscle in vitro suggest a role for NO as the neurotransmitter-mediated relaxation (Ignarro et al., 1990; Rajfer et al., 1992). There are two potential sources for endogenous nitric oxide in the penis: the nonadrenergic noncholinergic

(NANC) nerves and the endothelium of penile blood vessels and of the corporal tissue relaxation (Ignarro et al., 1990; Rajfer et al., 1992; Bush et al., 1992). In this study, transmural electrical stimulation produced frequency-dependent relaxation in the isolated rabbit corpus cavernosum pretreated with guanethidine and atropine. It has been reported that stimulation-induced relaxation of rabbit corporal smooth muscle is abolished by 3×10^{-7} M tetrodotoxin treatment and thus the response is regarded to result from nerve stimulation (Rajfer et al., 1992; Azadzo and Goldstein, 1992).

In recent years endothelial dysfunction of the corporal tissue has been recognised as an important cause of impotence. Impairment of endothelium-dependent relaxation may result in erectile dysfunction (Saenz de Tejada et al., 1989; Pickard et al., 1994; Azadzo and Saenz de Tejada, 1991, 1992). In previous studies, it has been shown that hypercholesterolemia (Azadzo and Saenz de Tejada, 1991) and diabetes mellitus (Azadzo and Saenz de Tejada, 1992), leading to damage of corporal endothelium, cause erectile dysfunction in the rabbit. Based on studies of several species, including human, NO, released by the endothelium, appears to play a major role in the initiation and maintenance of tumescence (Ignarro et al., 1990; Trigo-Rocha et al., 1993). The relaxation of human corporal smooth muscle in response to acetylcholine requires the presence of an intact endothelium (Saenz de Tejada et al., 1988). The observation in this study that hyperthyroidism impairs both neurogenic and endothelium-dependent relaxation of corporal smooth muscle, appears to be related to the alteration of the NO/cGMP pathway or hyperthyroidism may impair the relaxation of corporal smooth muscle or diminish its sensitivity to NO. However, these possibilities are unlikely since the corporal strips relaxed in response to sodium nitroprusside or papaverine. Since sodium

nitroprusside donates NO directly to smooth muscle (Moncada et al., 1991), NO causes the activation of guanylate cyclase and the intracellular accumulation of cGMP; this finding indicates that the smooth muscle response to NO is not altered by thyroid status. According to our knowledge, this result is the first *in vitro* evidence of impaired neurogenic and endothelium-dependent relaxant effect of the penile tissue with hyperthyroidism. At present, the mechanism underlying this impaired relaxation is not known; however, hyperthyroidism-induced dysfunction of corporal tissue does not appear to involve alterations in the cGMP-dependent relaxation of corpus cavernosum smooth muscle. It is also speculated that the decrease in the endothelium-dependent relaxation response to acetylcholine in hyperthyroid rabbits probably occurs at the level of the endothelium and not the smooth muscle cells and is most likely to be due to endothelial cell response to acetylcholine receptor-mediated activation. In addition, there were no differences in the KCl-induced contractile responses between two groups. Thus, the contractile mechanisms were intact in the cavernosal smooth muscle. It is also important to note that at the concentrations of phenylephrine used, developed tension was similar for corporal strips from euthyroid and hyperthyroid animals, thus ensuring that any difference in relaxation between hyperthyroid and euthyroid preparations was not due to differences in the degree of precontraction. Therefore, it is possible that hyperthyroidism impairs the synthesis or availability of NO in corpus cavernosum tissue. This is consistent with the results of previous reports on rats with hyperthyroidism. Authors reported that hyperthyroidism shows a reduction in basal NO synthesis/release; however, the responsiveness of the systemic circulation to NO is not altered in hyperthyroidism (Vargas et al., 1994).

Penis has the ability to synthesise various prostanoids and it has been suggested that arachidonic cascade products may be involved in the control of penile erection (Roy et al., 1984; Jeremy et al., 1986). The mechanism of impaired endothelium-dependent relaxation appears to be related to the increased production of a cyclooxygenase constrictor substance. Since, in the present study, we did not perform experiments with a cyclooxygenase inhibitor, such as indomethacin, we cannot draw any conclusions regarding the involvement of cyclooxygenase products at the present time. It is also known that hyperthyroidism causes an increase in the estradiol production rate, resulting in elevation of serum estradiol level (Chopra and Tulchinsky, 1974; Kidd et al., 1979). Studies with rat vascular smooth muscle cells have shown that 17 Beta-estradiol decreases nitric oxide synthase II synthesis (Zancan et al., 1999). Therefore, it is speculated that hyperthyroidis-induced impaired neurogenic- and/or endothelium-dependent relaxation may be due to the increase in estradiol production.

In conclusion, our findings suggest that hyperthyroidism may impair both neurogenic and endothelium-depen-

dent relaxation of corporal smooth muscle, leading to impotence. These results provide pharmacological evidence that hyperthyroidism affects corporal smooth muscle function at a presynaptic and postsynaptic level.

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