Neuronal Nitric Oxide Expression in Benign Prostatic Hyperplasia

BENİGN PROSTAT HİPERPLAZİSİNDE NÖRONAL NİTRİK OKSİT EKSPRESYONU

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Summary

Recently nitric oxide (NO) has been suggested to be a non-adrenergic non-cholinergic (NANC) neurotransmitter in the lower urinary tract. In this study we aimed to determine the location of the neuronal nitric oxide (nNOS) in the human prostate and compare the results with urinary retention and nonretention patients. nNOS immunohistochemistry were performed on histopatologically proven benign hyperplastic tissue. Patients were divided into two groups as a patients with urinary retention and non retention. nNOS staining was negative in all of patients with urinary retention, however, it was negative in 12 of 20 patients and weak positive in 8 in noncatheterized patients. Another finding was the negative staining in areas which bazal cell proliferation were seen, however strong staining were observed in the areas of containing atrophic glands. The pattern of glandular nNOS was relatively homogeneous in all specimens.

As a result, in patients with urinary retention, nNOS expression is reduced compared to that in noncatheterised patients. This study also shows that nitrinergic innervation is reduced in patients with urinary retention. This result may be the cause or result of urinary retention in BPH patients. Further studies are needed to confirm the role of nNOS in the pathophysiology of BPH.

Key Words: Nitric oxide, Benign prostatic hyperplasia, Urinary retention

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Özet

Son zamanlarda nitrik oksitin (NO) alt üriner traktta nonadrenerjik non-kolinerjik sistemin nörotransmitteri olduğu bildirilmiştir. Bu çalışmada insan prostatında nöronal nitrik oksitin (nNOS) lokalizasyonunu belirlemeyi ve sonuçları üriner retansiyon olan ve olmayanlarla karşılaştırmayı amaçladık. nNOS immünohistokimyası histopatolojik olarak benign prostat hiperplazisi teşhis edilen hastalarda yapıldı. Hastalar idrar retansiyonu olan ve olmayanlar şeklinde iki gruba ayrıldı. nNOS boyanması, idrar retansiyonu olan tüm hastalarda prostatik stromada negatifken retansiyon olmayan 20 hastanın 12'sinde negatif, 8'inde zayıf pozitif olarak bulundu. Bir diğer bulgu da bazal hücre proliferasyonu olan bölgelerde negatif boyanma varken atrofik gland içeren bölgelerde belirgin boyanma olması idi. Tüm dokularda glandüler nNOS rölatif olarak homojen bulundu.

Sonuç olarak üriner retansiyonlu hastalarda nNOS ekspresyonu retansiyonda olmayan hastalarla karşılaştırıldığında azalmış olarak bulundu. Bu çalışma aynı zamanda idrar retansiyonu olan hastalarda nitrinerjik innervasyonun azaldığını göstermektedir. Bu sonuç benign prostat hiperplazisi (BPH) olan hastalarda idrar retansiyonunun nedeni veya sonucu olabilir. BPH'nın patofizyolojisinde nNOS'un rolünü ortaya koyabilmek için daha fazla çalışmaya ihtiyaç olduğunu düşünüyoruz.

Anahtar Kelimeler: Nitrik oksit, Benign prostat hiperplazisi, Üriner retansiyon

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The rationale for alpha- blockers in the treatment of benign prostate hyperplasia (BPH) is based on the hypotesis that clinical BPH is, in part, due to prostate smooth-muscle mediated bladder outlet obstruction (1). Nitric oxide (NO) is an anorganic free-radical gas synthesized from L- arginine by a family of isoenzymes called NO synthases (NOS).

NO has been suggested to be a non-adrenergic non-cholinergic (NANC) neurotransmitter in the lower urinary tract in several species including man (2). It has also been suggested that nerve-induced

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release of NO mediates the relaxation of the bladder neck and urethra during the micturition reflex in humans (3). Hedlund et al reported that NO may act in the control of prostatic smooth muscle activity and secretion (4). Recent studies have shown that in obstructive BPH nitrinergic innervation is reduced compared to that in normal prostate tissue (5). Three different isoforms of NOSs have been suggested, neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS) (6). The aim of this study was to investigate the localisation of n NOS in the BPH tissue and compare the results between patients with urinary retention and not.

Materials and Methods

Patients

BPH specimens were obtained from 30 patients. Patients were divided into two groups those with catheterized because of urinary retention, mean age 64 years, and noncatheterized. Characteristics of noncatheterized patients have been shown in Table 1. No patient had received preoperative therapy because of obstructive symptoms. All specimens were obtained after open or transurethral prostatectomy.

Immunohistochemistry

For immunohistochemistry, the tissue sections were fixed in 10% buffered formalin for about 24 h, embedded in paraffin, and 5 µm sections were then deparaffinized. Then the sections were immersed in antigen retrival solution (Biogenex) and treated in microwave oven for 10 minutes. After cooling, the sections were washed with Phosphate Buffered Solution (PBS). The incubation with the primary antibody was done in a solution of 0.8% BSA and 20 mM NaN_3 in PBS containing the n NOS -specific mouse antibody (1:100 dilution) for 1 h at room temperature. After rinsing with PBS the sections were incubated with the secondary biotinylated goat antimouse antibody for 30 min at room temperature. Then, an alkalene phosphatase complex was utilized as a detection system (1:200 dilution) for another 1 hr. Finaly, the staining was developed for 15 min with fast red solution and counterstained with Mayer's hematoxylin. Negative controls sections were incubated in the absence of the primary antibody.

Table 1. Baseline Characteristic of noncatheterized patients (n=20)

Age (years) ± SD	64.2± 0.6
Maximum flow (ml/sec) ± SD	14.3 ± 3
Voiding detrusor pressure at maximum flow (cmH_2O) ± SD	34.2 ± 7
Residual urine (ml) ± SD	95.7 ± 25
Transrectal prostate volume (ml) ± SD	43.8 ± 17

The nNOS immunoreactivity of all specimens was evaluated blindly by two independent observers and rated according to a score which added a scale of intensity of staining to the area of staining in a high power field (score= intensity + area) (7). The intensity of staining was on the following scale: 0, no staining of nNOS in the prostate; 1+, mild staining; 2+, moderate staining; 3+, marked staining. The area of staining was evaluated as follows: 0, no staining of nNOS in the prostate in any microscopic field; 1+, <25% of the prostate stains positive; 2+, 25-50% staining positive; 3+, 50-75% staining positive; 4+, >75% staining positive. The maximum score when summed (area + intensity) was 7 and the minimum 0 (8).

Results

Immunohistochemical staining of nNOS was assessed in the glandular and stromal area of BPH tissues. In the stroma, a large amount of smooth muscle is present, there was negative nNOS staining in all of the patients with urethral catheter because of urinary retention. However, nNOS staining was negative in 12 of 20 patients and weak positive in 8 in the noncatheterized patients (Figure 1,2). Another prominent finding was the negative staining in areas which were seen bazal cell proliferation, however strong staining were observed in the areas of containing atrophic glands. The results of n NOS scoring has been shown in Table 2.

The pattern of glandular nNOS was relatively homogeneous in all specimens (Figure 3).

Discussion

There is increasing evidence suggesting that NO is a neurotransmitter substance for physiologically significant nonadrenergic, noncholinergic

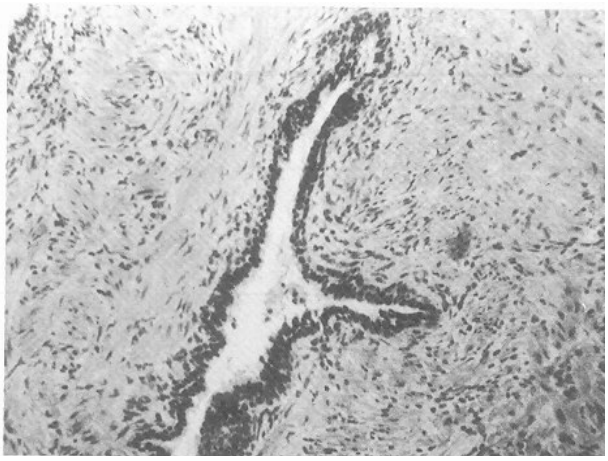

Figure 1. A large duct showing negative staining with nNOS (x 50).

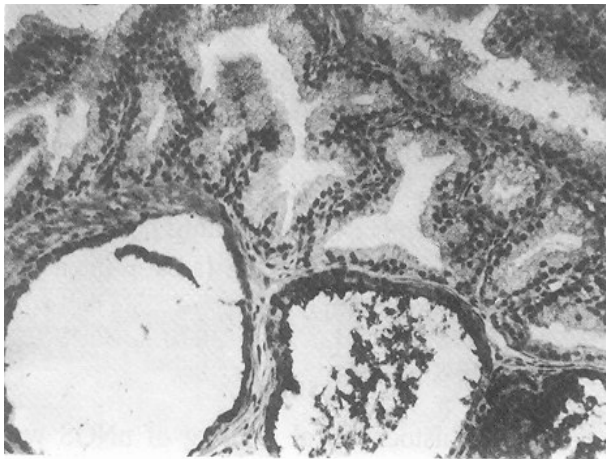

Figure 2. Atrophic prostatic glands exhibiting intense staining whereas hyperplastic glands with mild reaction (x 50).

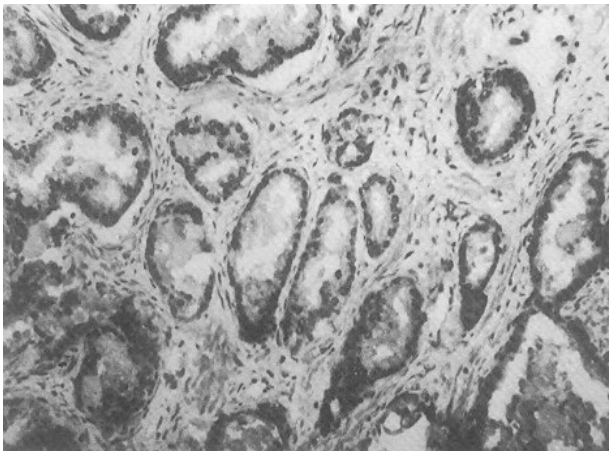

Figure 3. Hyperplastic glands showing moderate staining, stromal cells are negative (x 50).

Table 2. n NOS expression in BPH tissues

No of patients	Glandular	Stroma
Catheterized patients		
1	4	0
2	3	0
3	5	0
4	3	0
5	5	0
6	6	0
7	6	0
8	5	0
9	3	0
10	3	0
11	6	0
12	5	0
13	6	0
14	4	0
Noncatheterized patients		
1	5	1-2
2	6	1
3	4	0
4	5	1
5	5	0
6	5	0-1
7	4	1
8	4	0
9	5	0
10	4	0
11	5	0
12	5	0
13	6	2
14	5	0-1
15	4	0
16	5	0
17	5	0
18	5	2
19	4	0
20	6	0

(NANC) neuronal-mediated responses. Specifically, the L-Arg: NO pathway has been shown to play a significant role in mediating smooth muscle relaxation of several nongenitourinary tissues (9,10). Micturition is a urinary function mediated by the autonom nervous system. The role of NO as a mediator of normal and dysfunctional genitourinary smooth muscle activity is poorly understood. NANC relaxation of urethra has been recently shown to be mediated by NO (2,11). NOS positive nerves have been observed to course adjacent to the human prostate and penetrate into the peripheral prostate (12). These studies suggest that NO may

be a mediator of NNC neurotransmission in the human prostate. Recent studies have shown that NO is a NANC mediator of prostatic smooth muscle activity (4). The development of bladder outlet obstruction in men with BPH has been shown to be related to the area density of prostate smooth muscle (13). It is known that the tension of hyperplastic prostatic smooth muscle is mediated by alpha1 and endothelin agonists (14,15). It is well known that alpha1 blockers improves urinary flow rates and urinary symptoms in men with BPH (16). The pathophysiology of BPH is related to the loss of NO activity, resulting in greater baseline tension and bladder obstruction. Anderson et al reported that NO may contribute to relaxation of the outflow region (17). In another study Alm et al has shown that NOS nerves were very frequent in the smooth musculature of the urethra together with cholinergic, adrenergic and neuropeptide Y (NPY) -immunoreactive nerves. In their study outflow obstruction very overtly reduced the number of NOS-IR nerves in parallel with the general autonomic innervation (18). In view of these results we suggest that in prostate and presumeably in the bladder neck area NO may exert a transmitter role, both directly or by interaction with other transmitters.

Recently the presence of nNOS and nitrergic relaxation of the rabbit prostate have been reported (19). Previous experimental studies has shown that within the first 24 hours after acute complete urethral obstruction the initial bladder overdistension could lead to marked damage in bladder nerves in rabbits (20). Zhou et al has been reported that acute complete outlet obstruction causes neuronal degeneration in the intramural ganglia of the guinea pig urinary bladder (21). Similar changes may occur in case of urethral obstruction in prostate and this may further agrevate already occurred neuronal damage in BPH tissue. Bloch et al reported that NO plays an important role in the autonomic innervation of all compartment of prostate and in obstructive BPH the nitrinergic innervation is reduced compared to that in nonobstructed BPH (22).

The distribution of nNOS shows the importance of NO in the regulation of smooth muscle, blood flow and secretory function in the hyperplastic prostate. In patients with urinary retention due to BPH, the nitrinergic innervation is reduced compared to that in non catheterized patients. Uniformly negative staining of nNOS in patients with catheterized patients because of urinary retention may be cause or result of urinary retention in these patients. From view of these result we conclude that nNOS is reduced in patients with severe obstructive BPH patients. Further studies are needed to determine the role of nNOS in the pathophysiology of obstruction because of BPH.

REFERENCES

1. Caine M. The present role of alpha adrenergic blockers in the treatment of benign prostatic hypertrophy. J Urol 1986; 136: 1-6.

2. Andersson K-E, Garcia-Pascual A, Persson K, Forman A, Tottrup A. Electrically-induced nerve-mediated relaxation of rabbit urethra involves nitric oxide. J Urol 1992; 147: 253-9.

3. Leone AM, Wiklund NP, Hokfelt T, Brundin L, Moncada S. Release of nitric oxide by nerve stimulation in the human urogenital tract. Neuroreport 1994; 5: 733-

4. Hedlund P, Larsson B, Alm P, Anderson KE. Nitric oxide synthase-containig nerves and ganglia in the dog prostate: a comparison with other transmitter. Histochem 1996; 28(9):635-42.

5. Klotz T, Bloch W, Loch C, Engelmann U, Addicks K. Pattern of distribution of constituve isoforms of NO synthase in the normal prostate and obstructive prostatic hyperplasia. Urologe A 1997; 36(4): 318-22.

6. Forstermann U, Schmidt HHHW, Pollock JS, Sheng H, Mitchell JA, Warner TD, Nakane M, Murad F. Isoforms of nitric oxide synthase. Characterisation and purification from different cell types. Biochem Pharmacol 1991; 42: 1849-57.

7. Bredt DS, Snyder SH. Nitric oxide mediates glutamate-linked enhancement of c GMP levels in the cerebellum. Proc Natl Acad Sci USA 1989; 86: 9030-33.

8. Moochhala S, Chhatwal VJS, Chan STF, Ngoi SS, Chia YW, Rauff A. Nitric oxide synthase activity and expression in human colorectal cancer. Carcinogenesis 1996; 17: 1171-74.

9. Calignano A, Whittle BJ, Di Rosa M, Moncada S. Involvement of endogenous nitric oxide in the regulation rat intestinal mobility in vivo. Eur J Pharmacol 1992; 229: 273-6.

10. Shuttleworth CW, Sanders KM, Keef KD. Inhibition of nitric oxide synthesis reveals non-cholinergic excitatory neurotransmission in the canine proximal colon. Br J Pharmacol 1993; 109: 739-47.

11. Ignarro LJ, Bush PA, Buga GM, Woods KS, Fukuto JM, Rajfer J. Nitric oxide and cyclic GMP formation upon electrical field stimulation cause relaxation of corpus cavernosum smooth muscle. Biochem Biophys Res Commun 1990; 170: 843-50.

12.Burnett AL, Lowenstein CJ, Bredt DS, Chang TS, Snyder SH. Nitric oxide: a physiologic mediator of penile erection. Science 1992; 257: 401-3.

13.Shapiro E, Hartano V, Lepor H. The response to alpha blockade in benign prostatic hyperplasia is related to the percent area density of prostate smooth muscle. Prostate 1992; 21: 297-307 (15-4).

14.Lepor H, Tang R, Shapiro E. The alpha-adrenoceptor subtype mediating the tension of human prostatic smooth muscle. Prostate 1993; 22: 301-7.

15.Langenstroer P, Tang R, Shapiro E, Divish B, Opgenorth T, Lepor H. Endothelin-1 in the human prostate: tissue levels, source of production and isometrc tension studies. J Urol 1993; 149: 495-9.

16.Lepor H, Auerbach S, Puras-Baez A, Narayan P, Soloway M, Lowe F, Moon T, Leifer G, Madson P. A randomized placebo-controlled multicenter study of the efficacy and safety of terazosin in the treatment of benign prostatic hyperplasia. J Urol 1992: 148: 1467-74.

17.Tottrup A, Svane D, Forman A. Nitric oxide mediating NANC-inhibition in opossum lower esophageal sphincter. Am J Physiol 1991; 260: 385-9.

18.Alm P, Zygmunt PKE, Iselin C, Larsson B, Uvelius B, Werner S, Anderson KE. Nitric oxide synthase-immunoreactive, adrenergic, cholinergic, and peptidergic nerves of the female rat urinary tract; a comparative study. J Autonomic Nervuous System 1995; 56; 105-14.

19.Di Iulio JL, Li CG, Rand MJ. Determination of nitric oxide synthase activity in rat, pig and rabbit prostate glands. Eur J Pharmacol 1997; 337: 245-9.

20.Kitato S, Wein AJ, Kato K, Levin RM. Effect of acute complete obstruction on the rabbit urinary bladder. J Urol 1989; 141: 166-

21.Zhou Y, Ling EA. Effects of acute complete outlet obstruction on the NADPH-diaphorase reactivity in the intramural ganglia of the guinea pig urinary bladder: light and electron microscopic studies. J Urol 1997; 158: 916-23.

22.Bloch W, Klotz T, Loch C, Schmidt G, Engelmann U, Addicks K. Distribution of nitric oxide synthase implies a regulation of circulation, smooth muscle tone, and secre tory function in the human prostate by nitric oxide. Prostate 1997; 33: 1-8.