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<Original Article>

Bladder function in 17β-Estradiol Induced Nonbacterial Prostatitis Model of the Rat

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Abstract

Objectives: To investigated the bladder function of nonbacterial prostatitis (NBP) rat model that was induced by the injection of 17β-estradiol in castrated rats.

Methods: Ten-month-old male Wistar rats were divided into 2 groups (sham vs NBP; each N=8). NBP model was experimentally induced by castration followed by daily subcutaneous injection of 17β-estradiol for 30 days. On the 31st day after surgery, we investigated several parameters; (1) Voiding behavior, (2) Bladder blood flow, (3) Measurements of the prostate and bladder weight, the levels of proinflammatory cytokines (TNF-α and CXCL1) in the prostate and bladder, (4) Bladder contractile responses to electrical field stimulation (EFS), carbachol, and KCl.

Results: (1) Voiding behavior (average micturition volume, total urine volume and number of micturitions) and (2) bladder blood flow were not significantly different between sham and NBP group. (3) NBP led to a significant decrease in the prostatic weight and increase in proinflammatory cytokine levels in the prostate, while NBP did not cause a significant change in the bladder weight or proinflammatory cytokine levels in the bladder. (4) Bladder contractile forces in response to EFS, carbachol and KCl were not significantly different between sham and NBP group.

Conclusions: The present study suggests that NBP did not cause a significant change in the levels of proinflammatory cytokines in the bladder, and the bladder function is not influenced in this NBP model.

INTRODUCTION

Chronic inflammation in the prostate, chronic prostatitis (CP), has recently been recognized as an important component of the symptom progression of benign prostatic hyperplasia (BPH) and lower urinary tract symptoms (LUTS) [1]. Men with symptoms of chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS), NIH Category III [2], have commonly voiding complaints. The voiding symptoms do not correlate with urodynamic findings, but a significant proportion of the patients treated for CP/CPPS may have bladder outlet obstruction (BOO) or bladder dysfunction [3]. Numerous mechanistic explanations have been proposed for the association of human voiding dysfunctions and CP/CPPS. Therefore, the sequential relationship between prostatic inflammation and possible voiding dysfunctions remains unclear.

Although the many animal models presenting CP has been reported, there are few reports of those urodynamic change [4,5]. Bernoulli et al [4] used the noble rat model for nonbacterial prostatic inflammation (NBP) with the estradiol and testosterone 21-day releasing implants. They reported that urodynamic changes which developed in association with glandular inflammation indicated abnormal bladder function, reflecting an incipient obstruction. On the other hand, Funahashi et al [5] showed the rat chemical prostatic inflammation model induced by formalin injection into bilateral ventral lobes of the prostate exhibited bladder overactivity). In the present study, we investigated the bladder function of NBP rat model that was induced by the injection of 17β-estradiol in

castrated rats.

MATERIAL AND METHODS.

Animals

Ten-month-old male Wistar rats (Japan SLC, Hamamatsu, Japan) were housed five per cage in a room maintained at 20–26°C and 35–75% relative humidity with an alternating 12-hour light/dark cycle (the lights came on automatically at 8:00 a.m.). Food and water were freely given. All protocols were approved by Asahikawa Medical University Institutional Animal Care and Use Committee, and conducted in compliance with the Internal Regulations on Animal Experiments at Nippon Shinyaku Co., Ltd, which are based on the Law for the Humane Treatment and Management of Animals (Law No. 105, October 1, 1973, as revised on June 1, 2006).

Induction of Nonbacterial Prostatitis in Rats

Male Wistar rats were divided into 2 groups (sham-operated vs NBP; each N=8). The rats in sham-operated group underwent sham castration and daily injection with sesame oil vehicle. The rats in NBP groups were castrated and NBP was induced according to the method of Naslund et al [6]. Briefly, rats were injected subcutaneously with a daily dose of 0.25 mg/kg 17β-estradiol in 0.1 ml sesame oil for 30 days.

Voiding Behavior Recording

To measure the voiding behavior (number of micturitions, average

micturition volume, and total urine volume), each rat was placed in metabolic cages overnight for 12 hours on the 31st day after castration, where urine was directed into collectors on a GX-200 electronic balance (A and D, Tokyo, Japan). Urine output was monitored using a PowerLab® data acquisition system (PowerLab16/30, AD Instruments, New South Wales, Australia) connected to the electronic balance. Urine weight was converted to volume by assuming that 1 gm equals 1 ml. A stair-like weight change in the urine chart was deemed micturition.

Measurement of Bladder Blood Flow (BBF)

After micturition recording, each rat was anesthetized with pentobarbital (50 mg/kg intraperitoneally). The bladder was exposed and emptied by inserting a catheter through the urethra. After removing the catheter, the BBF was determined with a laser speckle blood flow imager (Omegazone OZ-1; Omegawave, Tokyo, Japan) as described by Oka et al [7]. For measurement, the bladder surface was diffusely illuminated with a 780-nm semiconductor laser, scattered light was filtered with a hybrid filter and detected by a charge-coupled-device (CCD) camera, and the images were transferred to a computer for analysis. Image pixels were analyzed to produce average perfusion values.

Determination of Inflammatory Markers

The procedures described by Sugimoto et al [9] were used to assay the proinflammatory cytokines (TNF-α and CXCL 1) in the bladder and prostate. In brief, a part of the bladder and prostate tissues were homogenized with a Physcotron Handy

Micro Homogenizer (Nition, Tokyo, Japan) in 10 volumes of ice-cold phosphate-buffered saline containing 200 mM phenylmethylsulfonyl fluoride as a protease inhibitor. The 10,000-g supernatant was further centrifuged at 30,000 g for 60 minutes and the resulting supernatant was used for the determination of cytokines with ELISA kits from R&D Systems (Minneapolis, MN, USA). Protein was determined with the Bio-Rad protein assay kit with bovine serum albumin as the standard.

Contractility Measurements in Isolated Bladder Smooth-Muscle Strips

After measurement of the BBF, the rats were sacrificed and the prostates and weight were rapidly removed and weighed. Contractile responses were measured as described by Oka et al [8]. The bladder dome was removed and full-thickness longitudinal strips of about 3 × 10 mm were taken from the bladder body. A part of the remaining bladder tissue was fixed overnight in 10% formaldehyde solution, then embedded in paraffin for histological studies. The rest of the tissue was rapidly frozen and stored at −80°C until further processing. Strips were placed in separate organ baths at 37°C containing Krebs–Henseleit buffer (142 mM NaCl, 23.8 mM $NaHCO_3$, 4.83 mM KCl, 0.96 mM KH_2PO_4, 1.20 mM $MgSO_4$, 12.5 mM HEPES, 5 mM glucose and 1.53 mM $CaCl_2$ adjusted to pH 7.4 at 37°C). The baths were aerated with a mixture of 95% O_2 and 5% CO_2. One end of each strip was connected to a model T7-8-240 force-displacement transducer (Orientec, Tokyo, Japan). Changes in muscle tension were measured and displayed on a Model RJG-4124 Recticorder chart recorder

connected to a Model AP-621G carrier amplifier (Nihon Kohden, Tokyo, Japan). Each strip was allowed to equilibrate for 60 minutes at 1 g resting tension. After equilibration, the strips were subjected to electric field stimulation (EFS) at 2, 8 and 32 Hz through 2 platinum electrodes set on either side of the muscle strip in the organ bath. Stimulation was applied in 10-second trains of 15-V square-wave pulses 1 millisecond in duration delivered with an SEN-7203 electronic stimulator (Nihon Kohden, Tokyo, Japan). After EFS, the maximal responses were determined sequentially for carbachol (100 μM) and KCl (100 mM). Responses were recorded isometrically and maximal tensions were compared.

Statistical Analysis

Data were analyzed by using the SAS program (SAS/STAT, Ver. 8.2, SAS Institute, Cary, NC). Differences between Sham-operated and NBP groups were analyzed for statistical significance by Student's *t*-test.

RESULTS.

Voiding behavior (number of micturitions, average micturition volume, and total urine volume) **(Figure 1)** and BBF (Sham: 71.34 ± 1.63 vs NBP: 74.17 ± 1.77 arbitrary units; n.s.) were not significantly different between sham-operated and NBP group.

Prostate weight after the 31-day period of treatment with 17β-estradiol was significantly decreased in the NBP group compared to the sham-operated group (Sham:

500.79 ± 15.99 vs NBP: 239.76 ± 20.39 mg; p<0.01), while NBP group did not cause a significant change in the bladder weight compared to the sham-operated group (Sham: 115.01 ± 11.17 vs NBP: 116.35 ± 2.17 mg; n.s.). **(Figure 2)**

The levels of the proinflammatory cytokines TNF-α and CXCL1 in the prostate, determined as a measure of prostate inflammation, were significantly increased by 2.0- and 11.5-fold, respectively, in the NBP group compared to the sham-operated group (TNF-α; Sham: 0.75 ± 0.18 vs NBP: 1.52 ± 0.18 pg/mg protein, CXCL1; Sham: 1.33 ± 0.26 vs NBP: 15.26 ± 1.50 pg/mg protein; p<0.01), while NBP did not cause a significant change in the levels of proinflammatory cytokines in the bladder **(Figure 3)**.

Bladder contractile forces in response to EFS (2, 8, and 32 Hz), carbachol and KCl were all tendency decreased in the NBP group compared to the sham-operated group by 91.7%, 88.1%, 87.3%, 74.6%, and 87.2%, respectively, and these were not significantly different between sham-operated and NBP group **(Figure 4)**.

DISCUSSION

Our data showed that the levels of the proinflammatory cytokines TNF-α and CXCL1 in the prostate were significantly increased in the NBP group compared to the sham-operated group, while NBP did not cause a significant change in the levels of proinflammatory cytokines in the bladder. Although the pathological examination was not done in this study, these results is presenting chronic inflammation of the prostate, not of

the bladder, in NBP model were same as previous reports [9]. And, the voiding behavior and BBF did not change in NBP rats. The blood flow of the prostate was not measured in this study, because the prostate from NBP rats was shrunk. But, Shabsigh et al reported castration resulted in a rapid and significant reduction of blood flow to the rat ventral prostate gland that was not seen in the bladder [11]. In this results, bladder contractile forces in response to EFS (2, 8, and 32 Hz), carbachol and KCl did not change in NBP rats. These results indicated the bladder function is not influenced in this NBP model.

Interestingly, Bernoulli et al [4] reported that urodynamic changes which developed in association with glandular inflammation indicated abnormal bladder function, reflecting an incipient obstruction, using the noble rat model for NBP with the estradiol and testosterone 21-day releasing implants. Even if it is called CP with the NBP model generally, the grade of prostatic inflammation changes with methods of creation. In fact, the grade of prostatic inflammation in their NBP model [4] is considered to have more severity compared with our model. Since, the bladder function in their NBP model is considered to have been influenced. And, it is thought that there is a possibility that the difference has appeared in the influence on a bladder function. The rat estradiol-treated castrated NBP model used in the present study shows severe inflammation and fibrosis in the lateral lobes of the prostate and a marked decrease in prostate mass, as reported by other investigators [6,9,12,13]. Naslund et al. [6] reported similar histopathological findings in estradiol-induced prostatitis in rats and CP in humans. The NBP model used

in the present study should therefore be suitable for investigating the treatment of human CP/CPPS.

On the other hand, Funahashi et al [5] showed the rat chemical prostatic inflammation model induced by formalin injection into bilateral ventral lobes of the prostate exhibited bladder overactivity as shown by frequent micturition. Their abstract is presenting chronic inflammation of the prostate, not of the bladder, in the chemical CP model. They reported that the proinflammatory cytokines, IL-1β mRNA, in the prostate in the chemical CP model was increased 3-fold compared to control rat. This level of IL-1β was similar the previous report in the NBP model [10]. Funahashi et al demonstrated that the prostate-to-bladder afferent cross-sensitization is a potential mechanism inducing irritative bladder symptoms in CP/CPPS. Probably, the severe inflammation of the prostate in this chemical CP model and it is considered that the prostate-to-bladder afferent cross-sensitization due to overlaps of nociceptive neurons within the spinal cord, so this is suggested in the previous paper [14].

In conclusion, the present study suggests that the bladder function is not influenced in this NBP model, and various rat prostatitis models differ in lower urinary tract dysfunction depending on the cause of prostatitis and severity grade.

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FIGURE LEGENDS

Figure 1. Effect on voiding behavior; (A) number of micturitions, (B) average micturition volume, and (C) total urine volume in NBP rats. Each column represents the mean ± S.E.

*; p<0.01 compared with sham-operated rats (Student's *t*-test).

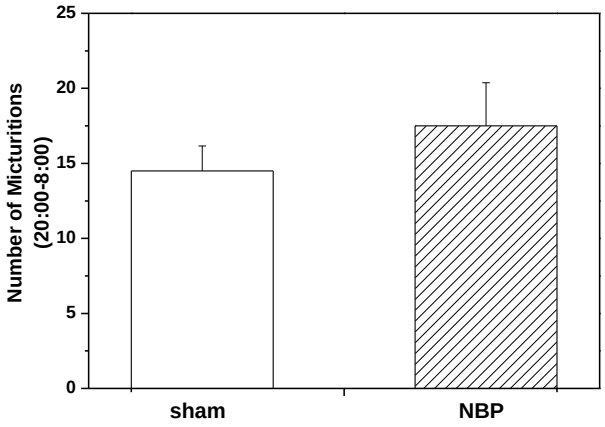
Figure 2. Effect on rat body, bladder and prostate weight in nonbacterial prostatitis (NBP) rats. Each column represents the mean ± S.E. *; p<0.01 compared with sham-operated rats (Student's *t*-test).

Figure 3. Effect on the proinflammatory cytokines; (A) TNF-α and (B) CXCL1 in the bladder and prostate in NBP rats, each column represents the mean ± S.E. *; p<0.01 compared with sham-operated rats (Student's *t*-test).

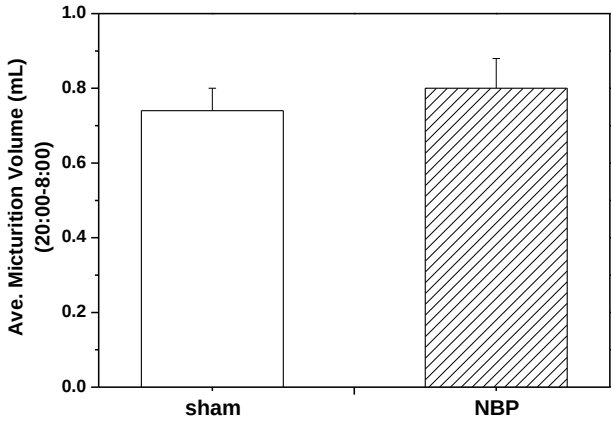
Figure 4. Effect on contractile responses to EFS (2, 8 and 32 Hz), carbachol (100 μM) and KCl (100 mM) in NBP rats. Each bar represents the mean ± S.E. *; p<0.01 compared with sham-operated rats (Student's *t*-test).

Figure 1

A


Number of Micturitions
(20:00-8:00)
25
20
15
10
5
0
sham
NBP

B


Ave. Micturition Volume (mL)
(20:00-8:00)
1.0
0.8
0.6
0.4
0.2
0.0
sham
NBP

C

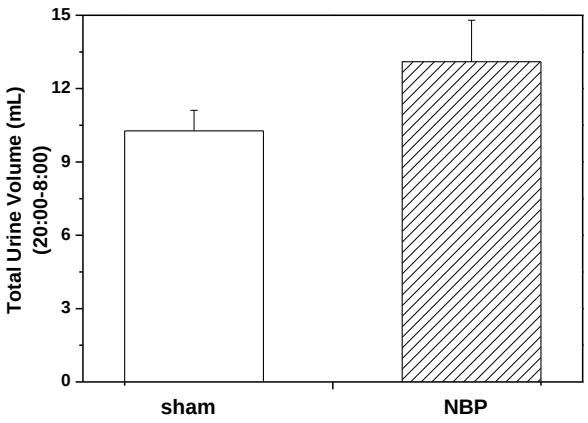

Total Urine Volume (mL)
(20:00-8:00)
15
12
9
6
3
0
sham
NBP

Figure 2

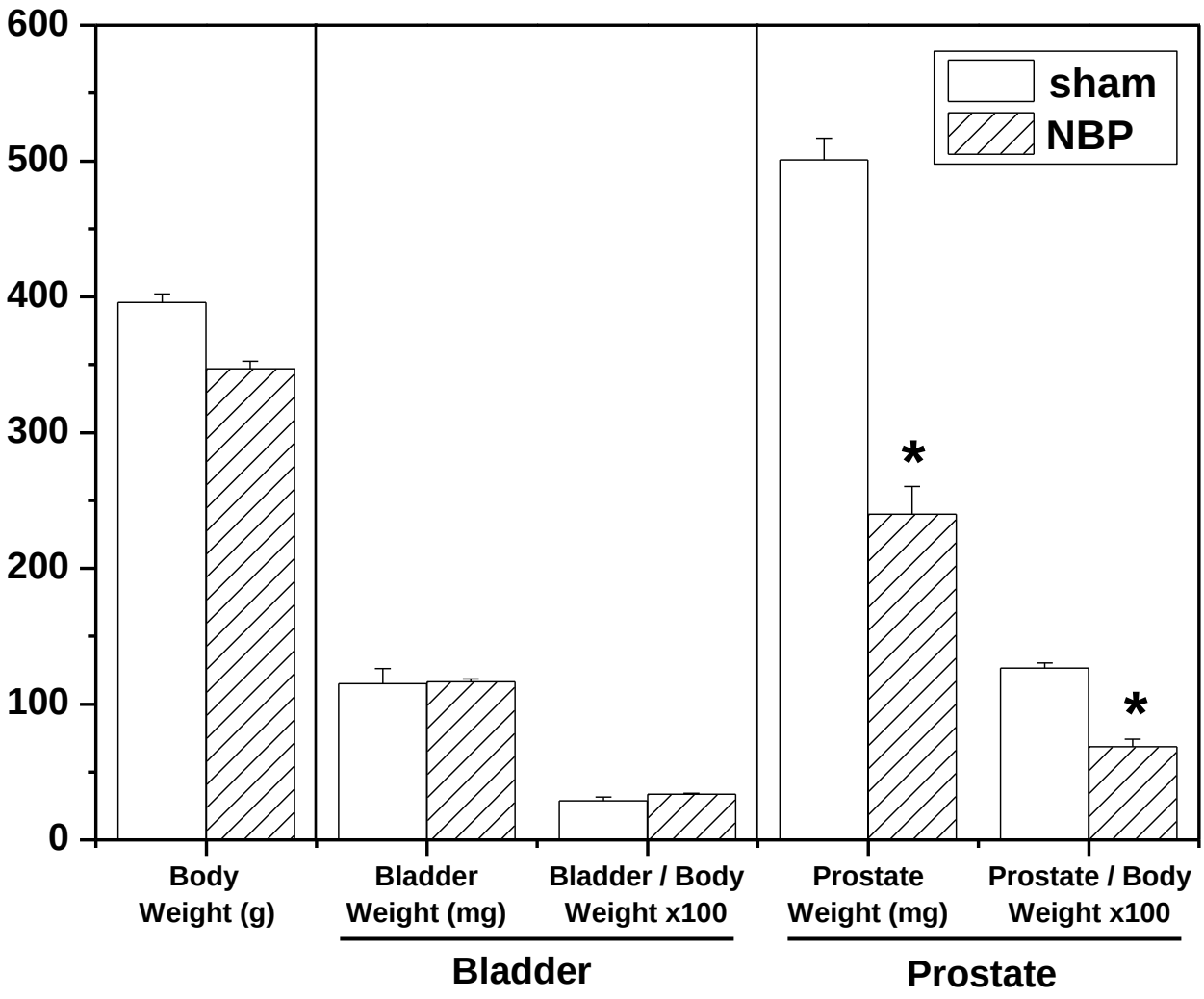
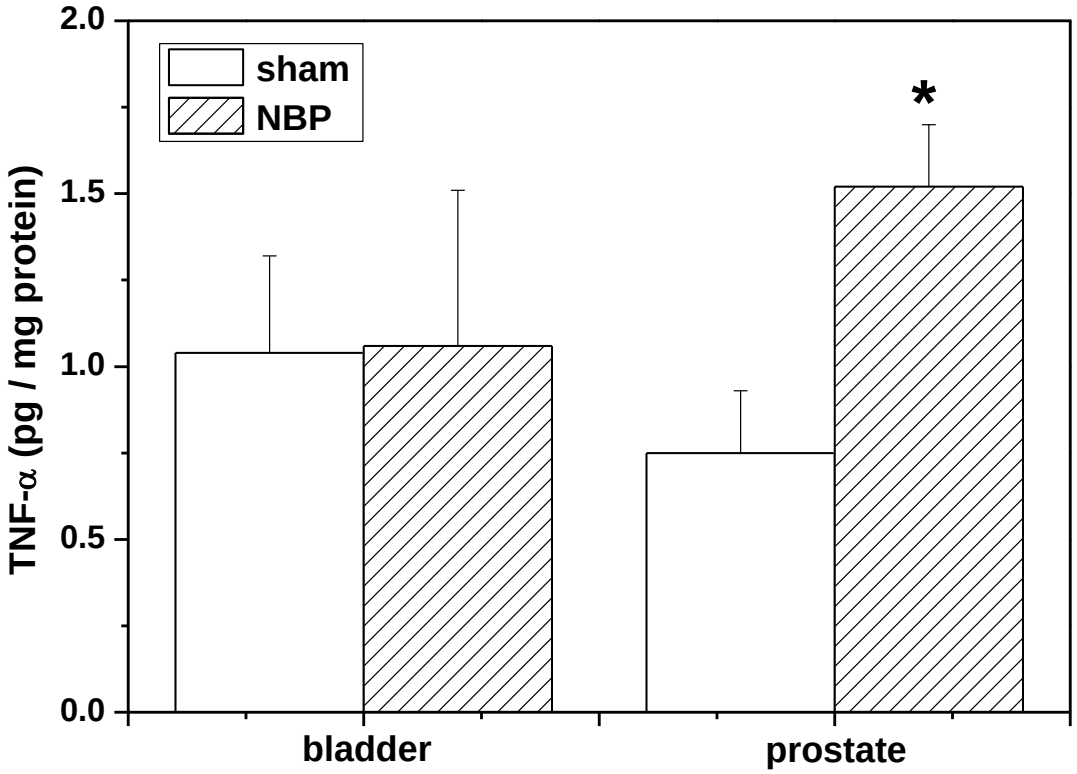

600
500
400
300
200
100
0
sham
NBP
*
*
Body Weight (g)
Bladder Weight (mg)
Bladder / Body Weight x100
Prostate Weight (mg)
Prostate / Body Weight x100
Bladder
Prostate

Figure 3

A


2.0
1.5
1.0
0.5
0.0
TNF-α (pg / mg protein)
sham
NBP
*
bladder
prostate

B

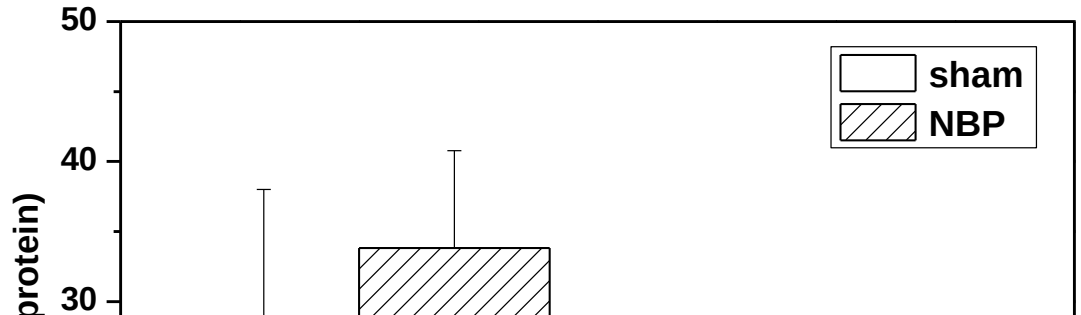
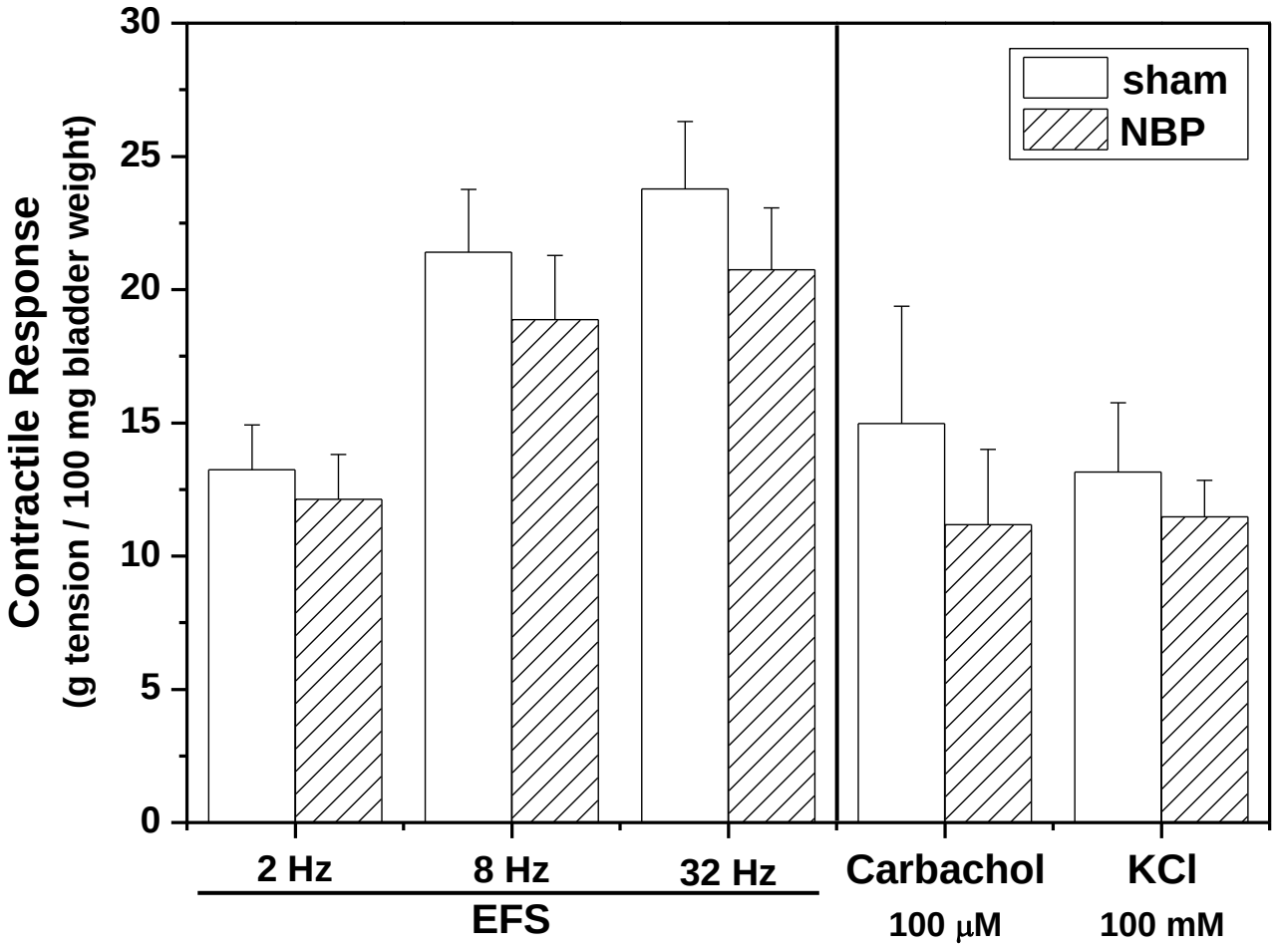

50
40
30
protein)
sham
NBP

Figure 4


Contractile Response
(g tension / 100 mg bladder weight)
30
25
20
15
10
5
0
sham
NBP
2 Hz
8 Hz
32 Hz
EFS
Carbachol
100 μM
KCl
100 mM