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The effect of hypercholesterolemia on the contractions of detrusor strips in response to neural stimulation in rats

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Aim: To investigate the effect of hypercholesterolemia on in vitro contractions of the detrusor smooth muscle stimulated by an electrical field in rats and the relative contributions of cholinergic and noncholinergic neurotransmission.

Materials and methods: Sprague-Dawley rats were fed with standard diet (NC group) or with 4% cholesterol diet (HC group). After 4 weeks, the urinary bladder was excised under anesthesia and 4 detrusor strips were prepared. Blood samples were collected for serum lipid profile, and aortic arches were dissected for histopathological examination. In vitro contractile function of the detrusor smooth muscle was evaluated with: 1) electrical field stimulation (EFS) only, 2) EFS in the presence of muscarinic antagonist atropine (10–6 M), and 3) EFS in the presence of atropine and P2X antagonist phosphate-6-azo (benzene-2,4-disulfonic acid) tetrasodium salt hydrate (10–4 M) or P2X agonist α,β-methylene-adenosine-5'-triphosphate (10–5 M).

Results: Plasma cholesterol was elevated in the HC group ($P < 0.05$); there was no sign of atherosclerosis. Although EFS-induced contractions were higher in the HC group, the difference was not significant. Cholinergic or purinergic antagonists decreased contractile response by desensitization with the purinergic agonist. The contribution of cholinergic and purinergic components were also similar in both groups.

Conclusion: The contribution of cholinergic and noncholinergic mechanisms of neurotransmission seems to be independent of the high cholesterol content of the diet and the plasma cholesterol level in rat detrusor muscle.

Key words: Cholesterol, cholinergic, urinary bladder, electrical field stimulation, purinergic

1. Introduction

Contraction of the urinary bladder via neural stimulation is mediated by cholinergic and nonadrenergic noncholinergic (NANC) mechanisms (1). Acetylcholine is the main mediator of bladder contraction via activation of muscarinic receptors (2). The contractile responses of detrusor strips elicited by electrical stimulation are not completely abolished by the muscarinic antagonist atropine. These atropine-resistant contractions are defined as NANC contractile components (3). The major NANC component is the purinergic system, which is involved both in sensory and motor functions of the urinary bladder (1). ATP released via neural stimulation is reported to be responsible for the initial rise in intravesical pressure and initiation of the micturition (4).

The biological properties of cell membranes are closely related to their cholesterol content. Dietary cholesterol is one of the sources of membrane cholesterol. Two-thirds of the cholesterol in the plasma is carried by low-density lipoprotein (LDL), which provides a source of cholesterol for the plasma membrane (5). The cholesterol-enriched domains of the cell membrane (6) play several important roles in cellular processes (7,8). They also provide a platform for G-protein, tyrosine kinase, and GTPase-mediated signaling in the detrusor membrane (9,10). Alterations in plasma cholesterol levels are closely related to membrane cholesterol, which in turn modulates signaling through the detrusor smooth muscle membrane (8–11). Based on this knowledge, it is natural to expect alterations of urinary bladder functions in response to changing plasma cholesterol.

The effect of hypercholesterolemia on detrusor contractility has been investigated in a limited number of studies (12–16). It has been shown that a number of neurotransmitters released in response to neural stimulation, which is mainly mediated by the cholinergic system and in addition by the purinergic system (17), are likely to be altered under pathological conditions

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(14,18,19). Since urinary bladder dysfunctions are increasing in incidence, especially in the elderly population where hypercholesterolemia is also common (20,21), we aimed to investigate the in vitro contractions of detrusor smooth muscle strips stimulated by an electrical field and the cholinergic and noncholinergic neurotransmission in hypercholesterolemic rats.

2. Materials and methods

The experimental protocol was approved by the Hacettepe University Institutional Ethics Committee for the Care and Use of Experimental Animals. Twelve male Sprague-Dawley rats of 4–7 weeks old were divided into control (n = 6) and cholesterol (n = 6) groups. The control group (NC) was fed with standard rat chow and the cholesterol group (HC) was fed with a high cholesterol diet (4% cholesterol and 0.2% deoxycholate) for 4 weeks (22,23). All rats were kept in standard conditions (room temperature in a 12-h light/12-h dark cycle) with free access to food and water. At the end of 4 weeks, rats were placed in metabolic cages for 24 h to determine food and water consumption, urine volume, and weight differences.

The rats were fasted overnight after being removed from the metabolic cages; the abdomen was then opened through a midline incision and the urinary bladder was excised under ether anesthesia. Blood samples were collected by cardiac puncture for the serum lipid profile. Aortic arches (1–1.5 cm) were dissected, removed, and fixed in 10% formalin for histopathological evaluation. In cold oxygenated Krebs solution, the urinary bladder was cleared of all the surrounding adipose and connective tissues and opened up with an incision starting from the bladder neck. Four longitudinal strips (10 × 2 mm) were prepared from the bladder body. Each strip was attached to a tension transducer (MAY FDT 05, Commat, Ankara, Turkey) and immersed in a 15-mL glass organ bath containing Krebs–Henseleit solution (118.4 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25.0 mM $NaHCO_3$, 2.5 mM $CaCl_2$, 12.2 mM glucose; pH 7.35–7.40) gassed with a mixture of 95% O_2 and 5% CO_2 and maintained at 37 °C under a resting tension of 0.5 g. Before any experimental procedures, the detrusor strips were allowed to equilibrate for at least 60 min of washing with fresh buffer every 15 min. Mechanical activity was recorded in real time by a data acquisition/analysis system (BIOPAC MP30, BIOPAC Systems Inc., Goleta, CA, USA). Each strip was subjected to the following contraction protocols. One strip was allocated as the time control.

2.1. Contraction protocols

The contraction protocols are summarized in Figure 1. In the first protocol, following equilibration, strips, suspended between 2 platinum electrodes, were stimulated by an electrical field stimulation (EFS). A series of square wave electrical pulses (0.15 ms pulse width, 100 V tension) of 5 s in duration was delivered at 3 min intervals (Grass stimulator; Model S88, Grass Institute, Quincy, MA, USA). The tension developed at 1, 2, 5, 10, 20, 40, and 80 Hz was recorded. After determining the frequency of maximal response, the strips were stimulated at the maximal response frequency twice at 3-min intervals. The EFS parameters were determined by modifying the suggestions of Longhurst and Uvelius (17).

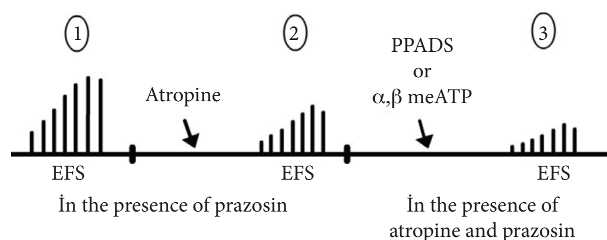

Figure 1. Schematic presentation of the experimental protocols applied to bladder strips: 1) EFS only, 2) EFS in the presence of atropine (10^{-6} M), and 3) EFS in the presence of atropine and PPADS (10^{-4} M) or α,β-meATP (10^{-5} M) to study the contraction of detrusor smooth muscle. Prazosin (10^{-7} M) was present throughout the EFS experiments.

The second protocol was applied after the strips were reequilibrated. EFS was repeated as described above after 20 min of incubation with muscarinic antagonist atropine (10^{-6} M).

In the third protocol, either P2X antagonist phosphate-6-azo (benzene-2,4-disulfonic acid) tetrasodium salt hydrate (PPADS) (10^{-4} M) (24) or P2X agonist α,β-methylene-adenosine-5'-triphosphate (α,β-meATP) (10^{-5} M) (25) was added to the bath in the presence of atropine (10^{-6} M) to isolate the noncholinergic component of the parasympathetic stimulation. EFS was repeated after an incubation period of 40 min for PPADS. α,β-meATP was administered repeatedly every 10 min to desensitize the detrusor strips until no contractile response was attained with the agonist. After 10 min of desensitization, EFS was repeated.

Prazosin (10^{-7} M) was present throughout the experiments to abolish α-adrenergic contractions that may be generated in response to EFS. In order to identify if direct stimulation of the detrusor muscle contributes to the contractile response, 1 μM tetrodotoxin was applied in the preliminary studies, which abolished all the contractile response to neural stimulation. Drugs and carriers were prepared freshly and added directly into the bath in order to not exceed the volume of the bath by more than 5%.

2.2. Serum lipid profile

Serum lipid profile (total cholesterol, triglyceride, high-density lipoprotein (HDL), LDL, and very-low-density

lipoprotein (VLDL)) was determined by diagnostic kits using a modular system autoanalyzer (Roche/Hitachi, Indianapolis, IN, USA) in the Clinical Biochemistry Laboratory of Hacettepe University Hospital.

2.3. Histopathological evaluation

Buffered formaldehyde (10%) was used to fix aortic arches. Routine light microscopic tissue processing methods were employed to process the samples. The sections were stained with hematoxylin-eosin and evaluated for atherosclerotic changes (26).

2.4. Drugs

Cholesterol, atropine sulfate, prazosin hydrochloride, PPADS, and α,β-meATP were purchased from Sigma-Aldrich (USA) and deoxycholate was purchased from Merck (Germany). Tetrodotoxin was kindly gifted by Professor Nuhan Puralı (Department of Biophysics, Faculty of Medicine, Hacettepe University). All drugs were dissolved and diluted in distilled water.

2.5. Analysis of data

Data were analyzed by BSL Pro Version 3.6.7 software (BIOPAC Systems Inc.). The force of contraction was normalized with wet tissue weight (g/100 mg wet tissue weight). The number of animals and strips were indicated by N and n, respectively. The inhibitory effects of drugs were calculated as the percent of maximal response of control strips.

Statistical analysis was performed with SPSS 15.0 (SPSS Inc., Chicago, IL, USA). The general metabolic properties, weight of the body and urinary bladder, and serum lipid profiles between groups were compared using Student's t-test for independent samples. EFS-induced frequency-response curves were compared by repeated measures analysis of variance (ANOVA) followed by Tukey's post hoc test. The effect of drugs was evaluated between groups at each frequency of EFS with Student's t-test. All data are presented as means ± SEM, and n represents the number of bladder strips. Statistical significance was considered at the level of $P < 0.05$.

3. Results

Body weight and metabolic cage results were similar between groups (Table 1). Total cholesterol and LDL levels were elevated in the cholesterol group compared with those of the control group ($P < 0.05$) (Table 2). Histological evaluation of aortic arch revealed no sign of atherosclerosis.

The results of first protocol revealed no significant difference in frequency response curves between the groups. EFS induced a frequency-dependent increase in contraction that reached maximum amplitude at 40 Hz stimulation in all detrusor strips. The mean of 3 contraction responses at 40 Hz was 25.1 ± 2.4 g/100 mg wet tissue weight in the NC group and 32.9±3.4 g/100 mg wet tissue weight in the HC group, and it did not achieve statistical significance.

The contribution of the cholinergic (atropine-sensitive) component was investigated in the second protocol. Frequency-response curves after incubation with atropine and prazosin were reduced in comparison to corresponding control curves in the NC and HC groups ($P < 0.05$) (Figure 2); however, there was no significant difference between groups. The proportion of cholinergic component to EFS-induced contractions increased in relation to frequency and the highest amplitude of contractions was observed at 40 Hz (38.1 ± 3.0% in the NC group and 36.9 ± 3.4% in the HC group) ($P > 0.05$) (Figure 3).

The contribution of the purinergic component was investigated via receptor blockage (P2X antagonist; PPADS) or desensitization (P2X agonist; α,β-meATP) in the third protocol. Desensitization with α,β-meATP was developed after the second administration. Contractile activity induced by EFS was significantly decreased in the presence of PPADS or α,β-meATP ($P < 0.05$) (Figure 2). The highest contribution of the purinergic component was

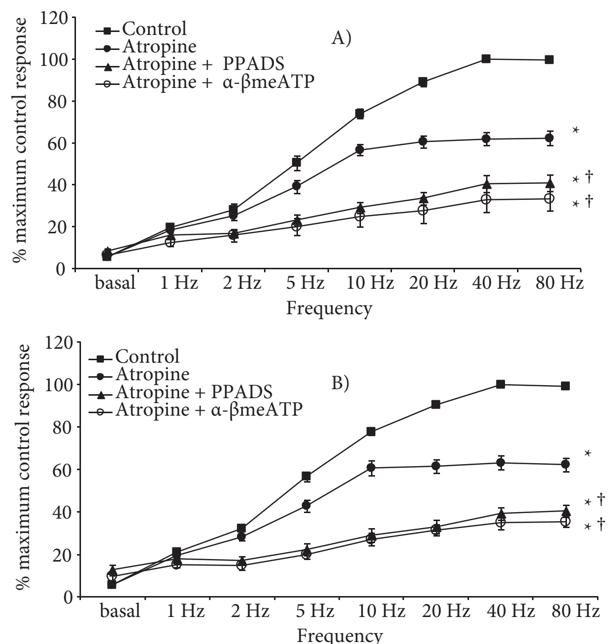

Figure 2. Contributions of cholinergic and purinergic components in contraction response of the rat detrusor smooth muscles to electrical field stimulation. Frequency-response curves in the absence (control; N/n= 6/24) and presence of either atropine (10^{-6} M; N/n = 6/18) alone or atropine + PPADS (10^{-4} M; N/n = 6/12) or atropine + α,β-meATP (10^{-5} M; N/n = 6/6) on bladders from A) control (NC) and B) cholesterol (HC) groups**.** The data are presented as the percentage of the maximum response in the control strips and values are presented as mean ± SEM.
***:** Significantly different compared to the control strips at $P < 0.05$; †: significantly different compared to atropine-applied strips at $P < 0.05$ (applicable for whole curves).

Table 1. Body weight and metabolic cage results of the control (NC) and cholesterol (HC) groups.

	NC (N = 6)	HC (N = 6)
Weight increase (g/4 weeks)	101.7 ± 19.5	141.7 ± 13.3
Food consumption (g/24 h)	20.3 ± 2.5	19.7 ± 2.5
Water consumption (mL/24 h)	43.3 ± 2.1	32.5 ± 3.1
Urine volume (mL/24 h)	16.5 ± 1.3	12.7 ± 0.8

Values are presented as mean ± SEM.

Table 2. Serum lipid profiles of the control (NC) and cholesterol (HC) groups.

	NC (mg/dL) (N = 6)	HC (mg/dL) (N = 6)
Total cholesterol	95.8 ± 3.7	132.7 ± 9.5*
Triglyceride	35.7 ± 2.0	29.5 ± 2.9
High density lipoprotein	72.8 ± 3.1	72.8 ± 3.1
Low density lipoprotein	28.2 ± 4.3	78.7 ± 9.2*
Very low density lipoprotein	7.1 ± 0.4	5.9 ± 0.6

Values are presented as mean ± SEM. *: Significantly different compared to the control group at $P < 0.05$.

observed at 10 Hz in the presence of PPADS (27.4 ± 2.0% in the NC group, 31.7 ± 3.6% in the HC group) or in the presence of α,β-meATP (31.8 ± 5% in the NC group, 33.5 ± 2.6% in the HC group) (Figure 3). No significant difference was observed in terms of the percentage of the purinergic components between the NC and HC groups.

Frequency response curves obtained from time-control strips were similar in all protocols both in the NC and the HC group.

4. Discussion

In the present study we investigated the effect of hypercholesterolemia on the contractile responses in isolated rat detrusor smooth muscle strips in vitro and the shares of the cholinergic and purinergic constituents. Our results indicate that both contractile response to neural stimulation and contribution of cholinergic and purinergic components remain relatively unchanged in hypercholesterolemic rats.

EFS causes stimulation of motor nerve endings embedded in the detrusor strips and results in frequency-dependent contraction. Under physiological conditions, the cholinergic component that can be eliminated by atropine has the largest share in detrusor smooth muscle contractions, and the purinergic component, although negligible in healthy human bladders, has a considerable impact in rats (19,27,28). The studies about the effect of hypercholesterolemia on detrusor smooth muscle contractility are limited and conflicting. Increased contractions in response to carbachol and EFS were reported in an atherosclerotic rabbit model developed by a high cholesterol diet (12). On the other hand, in a recent study, we found no difference in carbachol-induced contractions in hypercholesterolemic rats (23). Son et al. (14) showed no difference in the EFS-induced (50 Hz) contractile response in hypercholesterolemic rats that were

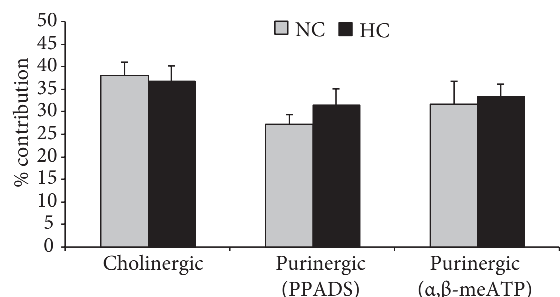

Figure 3. Contributions of cholinergic and purinergic (in the presence of PPADS (10^{-4} M) or α,β-meATP (10^{-5} M)) components in contraction response of the rat detrusor smooth muscles to EFS in the control (NC; N = 6) and cholesterol (HC; N = 6) groups.

also atherosclerotic. In line with this, in the present study we also showed that frequency contraction responses at all frequencies applied were comparable between groups. In our study the duration of the high cholesterol diet was sufficient to increase plasma cholesterol and LDL without causing atherosclerosis. The contribution of the cholinergic component in rat detrusor smooth muscle strips was reported as 42% (19), 47% (28), and 33% (27) in different studies. Similarly, we measured the cholinergic component as approximately 40% (field stimulation at 40 Hz) in both groups. Cholinergic and purinergic components are reported to change under pathological conditions affecting detrusor smooth muscles in, for example, diabetes (19), interstitial cystitis (18), unstable bladder (29), and overactive bladder (28). Yoshida et al. reported similar EFS responses in different age groups in human detrusor smooth muscle, but showed that the contribution of purinergic neurotransmission increases with age (30). Son et al. (14) reported no difference in response to EFS; however, they found a diminished proportion of the cholinergic component stimulated by EFS (50 Hz) in hyperlipidemic rats. We did not observe any difference in the atropine-sensitive nerve-mediated contractions between the groups in this study.

P2X receptors (mainly $P2X_1$), expressed in mammalian bladders, are ATP-gated ion channels that contribute to urinary bladder functions (24,28). Several studies suggested that PPADS strongly suppresses P2X-purinoceptor–mediated contractions (25,31,32). α,β-meATP, an ATP analog, is a selective P2X receptor agonist and resistant to ATPase. Thus, repeated application of α,β-meATP can desensitize the P2X receptors (24,25). In the present study, the purinergic component was found to be as ~30% (field stimulation at 10 Hz) in the presence of PPADS and ~33% (field stimulation at 10 and 20 Hz) in response to desensitization with α,β-meATP both in the NC and the HC group out of ~60% atropine-resistant contractions. Kennedy et al. showed 50% depression of atropine-resistant contractions with PPADS in guinea pig urinary bladder, in support of our results (24). Son et al. (14) reported an increased proportion of the purinergic component in the hypercholesterolemic group. Similarly, although insignificant, we also found an increase in the contribution of the purinergic component in the HC group. Rahman et al. (13) reported detrusor hypertrophy, up-regulation of P2X1, and increased expression of P2X3 receptors in the urinary bladder of hyperlipidemic rats compared to control group. These changes in purinergic receptors may be the possible cause of bladder overactivity. Since the above-mentioned data about hypercholesterolemia and detrusor function include prolonged periods of hypercholesterolemia (13,14), atherosclerosis, which intervenes with tissue oxygenation and nutrition, also complicates the picture in some of the studies (14). The results of these studies are not fully mirrored in our experimental conditions of 4 weeks of hypercholesterolemia with no sign of atherosclerosis. When cholinergic and purinergic components of EFS-induced contractile responses of detrusor smooth muscles were abolished, there remained a considerable amount of contraction (33% in NC group, 35% in HC group). Incomplete desensitization of P2X purinoceptors or the presence of other purinoceptors in the rat detrusor smooth muscle (24,33) could be involved in the residual contractile component together with the other NANC transmitter(s) released (18,34–36).

Hypercholesterolemia, due to the alterations of membrane structure and signaling mechanisms in relation to it, is a good candidate to be listed among the other pathological conditions (14,18,19) where the share of the atropine-resistant detrusor smooth muscle contractions is subject to change. Although the results of this study suggest no change in neurotransmission in detrusor smooth muscles in hypercholesterolemic rats, we strongly suggest further studies in different species and/or longer durations of hypercholesterolemia, since the role of the purinergic component in the contraction of the detrusor smooth muscle, which may have clinical relevance, remains to be established.

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