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The Effects of 1,1-Dimethylbiguanide Hydrochloride (Metformin) on Detrusor Muscle Contractile Response in Ovariectomized Female Rats

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ABSTRACT

Aim: Menopause is defined as the depletion of the ovarian follicular reserve followed by the cessation of menstrual cycles. It has been reported that gonadal steroid hormones play an important role in bladder function in women. Changes in urine pattern including overactive bladder, stress incontinence and recurrent urinary tract infections occur as a result of menopause. 1,1-dimethylbiguanide hydrochloride, metformin, (MET) is an oral anti-diabetic drug used to reduce hepatic glucose production and peripheral insulin resistance. Recent studies have revealed that MET has a protective effects in diabetes induced bladder dysfunction. The aim of this study was to test the therapeutic potential of MET in detrusor contractile function of ovariectomized (OVX) female rats.

Material and Methods: Bilateral ovariectomy was performed to eliminate endogenous gonadal steroids secretion. Four groups are designed with 8 animals in each group: Control, MET-administered control, OVX, and MET-administered OVX groups. MET (25 mg/kg) was administered daily by oral gavage for 14 days. Contractile activity of isolated bladder muscle strips were evaluated in vitro organ bath. The contractile responses of detrusor strips were determined using different doses of carbachol (10-8-10-2M) and purinergic agonist ATP. The relaxation response of strips were determined by isoproterenol

Results: The contractile responses of detrusor muscle strips to carbachol at doses 10-5-10-2 M were decreased in the OVX group compared to control and MET treated control groups. MET treatment partially reversed the reduction in OVX-induced contractile responses at 10-2 and 10 -3 M carbachol doses. There were no statistically significant difference in relaxation response between the experimental groups.

Conclusion: Our findings suggest that treatment with MET could be the new potential therapeutic agent against bladder dyfunction in postmenopausal women. Further studies are needed for the therapeutic potential of MET in detrusor dysfunction induced by menopause.

Keywords: *Ovariectomy, Detrusor contractility, Menopause, Metformin*

Ovarektomize Dişi Sıçanlarda 1,1-Dimetilbiguanit Hidroklorid (Metformin)'in Detrüsör Kas Kontraktil Yanıtı Üzerine Etkileri

ÖZ

Amaç: Menopoz, yumurtalık foliküler rezervinin tükenmesi ve ardından adet döngüsünün durması olarak tanımlanır. Kadınlarda gonadal steroid hormonlarının mesane fonksiyonunda önemli rol oynadığı bildirilmiştir. Menopozun bir sonucu olarak idrar düzeninde değişiklikler meydana gelir. 1,1-dimetilbiguanid hidroklorür, metformin (MET), hepatik glukoz üretimini ve periferik insülin direncini azaltmak için kullanılan oral bir anti-diyabetik ilaçtır. Son zamanlarda yapılan çalışmalar, MET'in diyabetin neden olduğu mesane disfonksiyonunda koruyucu etkileri olduğunu ortaya koymuştur. Bu çalışmanın amacı, yumurtalıkları alınmış (OVX) dişi sıçanların detrusor kasılma fonksiyonunda MET'nin terapötik potansiyelini değerlendirmektir.

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Gereç ve Yöntemler: Endojen gonadal steroid sekresyonunu ortadan kaldırmak için bilateral ovariektomi yapıldı. Her grupta 8 hayvan olacak şekilde dört grup tasarlanmıştır: Kontrol, MET uygulanan kontrol, OVX ve MET uygulanan OVX grupları. MET (25 mg/kg) 14 gün boyunca oral gavaj yoluyla günlük olarak uygulandı. İzole mesane kas şeritlerinin kontraktil aktivitesi in vitro organ banyosunda değerlendirildi. Detrusor şeritlerinin kasılma yanıtları, farklı dozlarda karbakol (10-8-10-2M) ve purinerjik agonist ATP kullanılarak belirlendi. Striplerin gevşeme yanıtı izoproterenol ile değerlendirildi.

Bulgular: Detrusor kas şeritlerinin 10-5-10-2 M dozlarda karbakole olan kontraktil yanıtları, OVX grubunda, kontrol ve MET ile tedavi edilen kontrol gruplarına kıyasla azaldı. MET tedavisi, 10-2 ve 10-3 M karbakol dozlarında OVX'in neden olduğu kasılma cevaplarındaki azalmayı kısmen tersine çevirdi. Deney grupları arasında gevşeme yanıtında istatistiksel olarak anlamlı bir fark yoktu.

Sonuç: Bulgularımız, MET ile tedavinin postmenopozal kadınlarda mesane fonksiyon bozukluğuna karşı yeni potansiyel terapötik ajan olabileceğini düşündürmektedir. Menopozun neden olduğu detrusor disfonksiyonunda MET'nin terapötik potansiyeli için daha ileri çalışmalara ihtiyaç vardır.

Anahtar Sözcükler: *Ovarektomi, Detrusör kontraktilitesi, Menopoz, Metformin*

INTRODUCTION

Menopause is resulted by depletion of ovarian follicular activity, followed by cessation of menstrual cycles. Most of women may experience a derangement of the endocrine system and related symptoms due to lack of gonadal steroid hormones during the transition to menopause (1). Voiding pattern changes take place during the menopause period (2). Various urinary problems such as overactive bladder, increased frequency, detrusor overactivity, and recurrent infections can occur during menopause (3).

Urinary tract has estrogen receptors and are sensitive to hormonal changes that occur during the menopause. Thus, symptoms associated with menopause include dysuria, urinary frequency, and a range of urinary disorders such as recurrent bladder infections that may contribute to the development of overactive bladder syndrome (OABS) (2, 4). Menopause is a risk factor for OABS, with its prevalence increasing significantly after the age of 40, reaching 39% in women aged 76 (5). It has been proposed that estrogen deficiency plays an important role in the genesis OABS because hypoestrogenism can induce alterations both in muscle fibers and in the innervation of the bladder. Indeed, it has been reported that menopause causes changes in the density of sensory nerve fibers in the bladder, thus triggering aberrant contractile activity similar to bladder hyperactivity (5). Ovariectomized (OVX) rats are often used as animal models of postmenopausal period. Voiding pattern alterations in ovariectomized rats was seen to related with estrogen deficiency (2,6,7). Previous studies showed that gonadal steroid deficiency results in reduced blood flow to bladder, enhanced bladder smooth muscle atrophy and collagen content, and decreased detrusor contractile responses to agonists (8). Estrogen replacement therapy is often used for relieving the symptoms of OABS in postmenopausal women (9). However, recent studies have shown that hormone replacement therapy (HRT) can increase the incidence of urinary incontinence in menopausal women (10).

1,1-Dimethylbiguanide hydrochloride, metformin (MET), is an anti-diabetic drug generally used for treatment of type II diabetes, which has been regarded to exert the neuroprotective through the anti-inflammatory (11) and antioxidant (12) actions. Recent studies have shown that MET has a protective effect against cyclophosphamide-induced cystitis in mice (13). Bladder dysfunction is very common in diabetes mellitus (14) and obesity (15). Various clinical studies have shown that bladder dysfunctions in diabetic patients, such as detrusor overactivity, impaired detrusor contractility and reflex activity (14).

Anti-muscarinics or adrenergic β3- receptor agonists are used as primary option for treatment of OABS. However, antimuscarinic drugs are not selective for bladder, in addition to low tolerability in patients by side effects, makes the levels of clinical response to be weak (16). Therefore, it is necessary to search for new more effective therapeutic strategies that can be better tolerated by patients. Nevertheless, the effects of MET in contractile responses of detrusor muscle have not been reported yet in ovariectomized rats. Based on the above information, the aim of our study was to determine the effect of MET treatment on the contractile response of isolated bladder strips in OVX rats.

MATERIAL and METHODS

Animals

Female Wistar Albino rats aged 3-4 months were used. They were housed in pairs in a temperature and humidity-controlled room on a 12:12 h reverse light-dark cycle. All rats were given free access to food and water ad libitum. All experimental procedures were approved by the Zonguldak Bulent Ecevit University Animal Care and Use Ethical Committee (ethical approve number: 2020-04-06/02)

Ovariectomy Procedure

Bilateral ovariectomy was done under mixture of ketamine (90 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) anesthesia to remove endogenous ovarian hormone secretion. Ovariectomy was performed at 14 days before to the MET treatment. All surgical procedures on the animals were conducted aseptic conditions as previously explained (17). Bilateral ovariectomy was done through dorsal incision. The rats in the sham groups subjected the same surgical procedure without removal of the ovaries.

Experimental Groups

Four groups were formed, with a total of 32 animals: Control group consisted of sham operated grup received daily saline (n=8). The control+MET-treated group consisted of sham-operated female rats treated with MET (25 mg/kg, by gavage) (n=8). OVX group consisted ovariectomized rats and received daily saline (n=8). The OVX+MET treated group consisted of ovariectomized rats treated with MET (25 mg/kg, via gavage) for 14 days (n=8). MET was started 14 days after the ovariectomy procedure. Metformin hydrochloride (Glucophage, Merck Serono Drug) was administered by orally at doses of 25 mg/kg for 14 days (12).

Animals were sacrificed after 14 days of MET treatment by high dose of anesthesia. Bladder tissues were isolated and the contractile activity of the bladder strips were evaluated in vitro.

Bladder Strips Contractile Activity

Whole urinary bladder was isolated and the detrusor muscle strips with intact urothelium (detrusor and mucosa) were cut 2 x 15 mm long. Strips were suspended under 2 g load in 20 mL organ bath filled with warmed (37 °C), oxygenated (95% O_2 and 5% CO_2) Krebs solution (in mmol L^{-1}: NaCl, 118.5; KCl, 4.8; KH_2PO_4, 1.2; $MgSO_4 \cdot 7H_2O$, 1.2; $CaCl_2$ 1.9; $NaHCO_3$, 25; glucose, 10.1). Isometric contractile force were monitered using a Software (MP30 Biopac Systems Inc, Santa Barbara, CA, USA). Detrusor strip was mounted to organ bath longitidunally with 4/0 silk ligatures between two curved hooks. One end of strip was connected to a the lower hook was fixed to the bottom of the bath and the other to a physiologic data acquisition system (MP30 Biopac Systems Inc, Santa Barbara, CA, USA). Organ bath medium was changed every 15 min during in vitro experimets. After 60 min equilibration period at 2 g load, potassium chloride (KCl) was added to the organ chamber to a final concentration of 80 mM before the beginning of each experiment. The contraction recorded in response to KCl was considered as the reference response (18). After washing 3 times, muscarinic agonist carbachol (CCh) was cumulatively added to the organ bath at final various concentrations ranging from 10^{-7} to 10^{-2} $molL^{-1}$.Contractile response of strips were expresses as %contracility of their KCl responses. The contactile response to ATP (10mM) was also determined in detrusor strips. The Krebs solution in organ bath was changed every 15 min during the experiment. The relaxation response of strips were determined by response to isoproterenol. After resting period again, strips were contracted with $3X10^{-6}$ M of CCh and then added cumulative concentration of isoproterenol (10^{-9}-10^{-2} M). Isoproterenone relaxation response were estimated as %relaxation of the strip contraction response to $3X10^{-6}$ M dose of CCh (18). At the end of each contractile response experiment, the weights of strips were measured.

Statistical Analysis

The data were represented as mean ± standard error of mean (SEM). All data were evaluated by non-parametric Kruskal-Wallis test followed by Dunn test to find out the significance of post hoc comparisons by using SPSS ver. 21.0 package program. It was considered statistically significant when the p value was less than 0.05.

RESULTS

Bladder Weights

The wet weight of bladder of experimental groups are shown in Table 1. The wet weight of bladders was determined significantly decreased after ovariectomy (0.14±0.008 g) compared with control groups (0.24±0.09 g). The bladder wet weigh of MET treated OVX group (0.22±0.04 g) was observed heavier than OVX group, however it was not statistically significant.

Contractile Response of Bladder Strips

Carbachol (10^{-9}-10^{-2} M) ATP (10 mMl) induced concentration-dependent contractile responses in the detrusor strips of the rat are shown Figure 1 and Figure 2, respectively. Cumulative carbachol responses were analyzed in order to

Table 1: Effect of ovariectomy and MET treatment on the wet weight of bladder of rats. The wet weight of bladder tissue was decreased after ovariectomy. Values are presented as means±S.E.M., $n = 8$ each group. • $p < 0.05$, as compared OVX group and ▪MET-treated control group.

Groups	Bladder weight
Control	0.24±0.09
Control+MET	0.25±0.12
OVX	0.14±0.008▪•
OVX+MET	0.22±0.04

evaluate the effects of ovariectomy and MET treatment in receptor-mediated contractions of detrusor smooth muscle. Contraction responses to carbachol doses were expressed as a percentage of contraction responses induced by KCI. The contractile responses of detrusor muscle strips to carbachol at doses 10^{-5}-10^{-2} M were decreased in the OVX group (128±8.45;142±9.48;156±11.43; 155±16.54) compared to control (215±16.15; 289±19.51; 289±31.37; 319±37.88) and MET treated control groups (212±21.1; 243±15.63; 254±15.94; 273±12.63). MET treatment partially reversed the reduction in OVX-induced contractile responses at 10^{-2} and 10^{-3} M carbachol doses. The contractile response to purinergic receptor agonist ATP did not significantly change between all experimental groups (Figure 2).

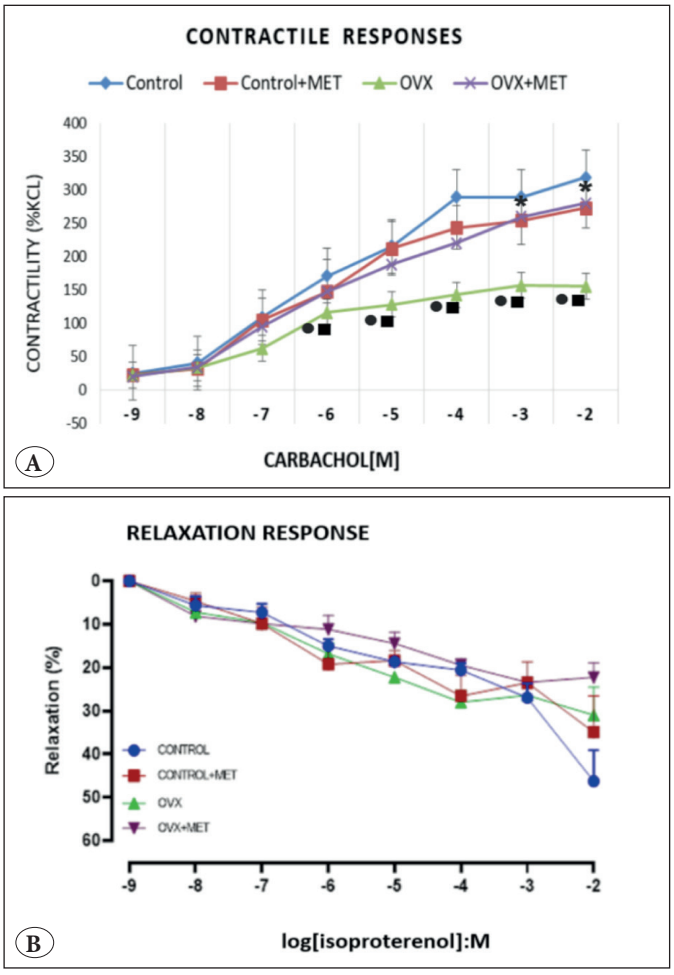

Figure 1: Concentration-response curves were obtained **(A)** by cumulative addition of carbachol (10^{-9}-10^{-2} M) and **(B)**relaxation response to isoproterenol (10^{-9}-10^{-2} M) to organ bath. Carbachol-induced contractile responses were significantly decreased in OVX group (Panel A). The contractile responses to carbachol were restored with MET treatment at at 10^{-2} and 10^{-3} doses of carbachol. Relaxation responses to isoproterenole were not different between experimental groups (Panel B).
Values are presented as means±S.E.M., n = 8 each group. Significant difference versus the • control and ▪ MET treated control groups, * significant difference versus the OVX group ($p < 0.05$).

Relaxation response of bladder strips were also evaluated in organ bath experiments. Relaxation responses were obtained by applying isoproterenol (10^{-5}-10^{-2}) to the detrusor muscle strips pre-contracted with carbachol (3×10^{-6}). There was no statistically significant difference in relaxation responses between the experimental groups (Figure 1, Panel B).

DISCUSSION

In this study, it was aimed to demonstrate that contractile response of detrusor muscle to muscarinic activation decreased after ovariectomy. In addition, it was analyzed the effect of MET on ovariectomy induced bladder contractile dysfunction in rats. MET treatment partially improved contractile dysfunction of detrusor muscle after ovariectomy.

Menopause is the end of the reproductive period induced by a woman's nature or by bilateral oophorectomy (19). The decrease in estrogen concentration associated with menopause is considered to be the main factor responsible for the increased prevalence of urinary incontinence in women (10). Sex hormones are known to have an important influence on the function of the lower urinary tract throughout adult life. Menopause increases risk of overactive bladder and its prevalence also increases with older age, especially after menopause (20). Estrogen receptors are found in the urinary tract and estrogens play a role for regulating urinary tract functions (21). It was reported that estrogen receptors are found in the epithelial cells of the bladder, trigone, urethra, and pelvic floor (4). During menopause, estrogen deficiency contributes to the atrophy of the lower urinary tract and pelvic floor and induces urinary tract symptoms such

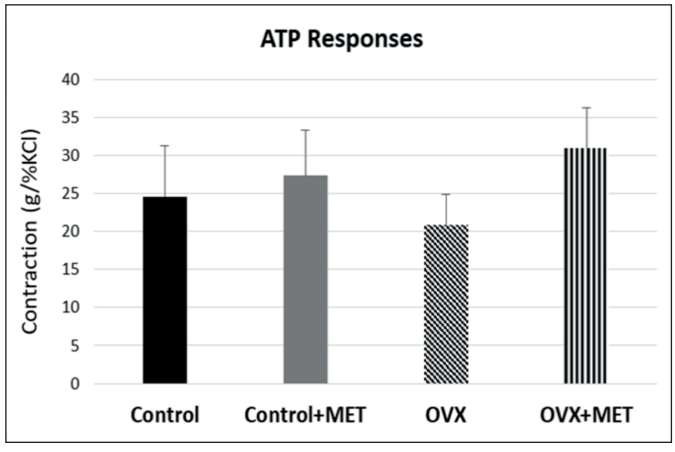

Figure 2: ATP responses of bladder strips. The contractile response of the strips to ATP was not altered by either ovariectomy or MET treatment.

as increased frequency, incontinence, urgency and nocturia. The relationship between overactive bladder and menopause is not clear but the decrease in ovarian hormones affect both in muscle fibers and innervation of bladder tissue (5). Ovariectomy causes bladder mucosa and detrusor smooth muscle atrophy, reduced blood flow, decreased bladder compliance and contractility (20,21). Changes in voiding patterns in ovariectomized rats have been associated with gonadal hormone deficiency (6). It was found that ovariectomy caused nerve damage and decreased acetylcholine (Ach) release form nerve terminals which contribute voiding dysfunction (20). It is also noted that the number of interstitial cells of the Cajal are reduced after the rat surgical menopause model (21). Thus, HRT is used in postmenopausal women with urinary tract dysfunction (10). HRT can considerably affect the bladder function by changing detrusor innervation and vascular density and distribution of receptor density, smooth muscle size and connective tissue density (22,23). At the same time, HRT is reported to improve the contractile response of the detrusor to carbachol (23). Rehfuss et al. (24) reported that the low estrogen level induced decreased bladder mass, smooth muscle content, contraction, blood fl ow and increased free radical damage. Also, previous studies suggested that OVX can cause a decreased of blood flow around the bladder neck and urethra, mucosal and smooth muscle atrophy, increased collagen synthesis and distribution, and reduced contractility. We found that the contraction induced by the muscarinic receptor agonist carbachol was significantly reduced in the detrusor muscle strips of OVX rats, consistent with previous studies (2,5,7,23). Ach and ATP are the main neurotransmitters that contract the detrusor muscle. The receptors for Ach (M2 and M3) and ATP(P2X1) present in the detrusor smooth muscle (25). The release of Ach from cholinergic fibers and its binding with muscarinic receptors constitute the major mechanism of detrusor contraction (26). Many investigators have demonstrated the downregulation of muscarinic receptors in the urinary bladder following ovariectomy in animals (23). It has also been postulated that an increase in collagen deposition, and impairment of Ach release may contribute contractile dysfunction after ovariectomy (20,23). We found that the in vitro detrusor muscle strips contractile acvivity in OVX rats were significantly reduced compared with conrol groups. This decreased contractile response to carbachol may be attributed to due impairment of contractile machinery of detrusor muscle after ovariectomy. However, treatment with MET at dose of 25 mg/kg partially improved contractile response of detrusor muscle of OVX rats. MET is widely used for the treatment of type II diabetes to reduce blood glucose level. MET also has diversity of pleiotropic effects such as, anti-inflammatory, antioxidant, anti-cancer effects (12,27-29). Several studies have reported that the AMP-activated protein kinase (AMPK) and its downstream signaling pathway are involved in these protective effects (29). It has been demonstrated that AMPK is an important modulator of glucose metabolism, inflammatory response, oxidative stress and smooth muscle contraction (29,30). In this study, MET treatment increased the sensitivity of bladder contraction to cholinergic agonists. MET may have ameliorated the detrusor contraction disorder caused by OVX through AMPK involved in smooth muscle contraction. Bladder dysfunction is a common diabetes complication affecting 80% of patients with diabetes and causes a range of voiding and storage symptoms (14). Several studies have reported that diabetes might lead to the bladder dysfunction by changing the innervation and vasculature of the bladder. Wang et al. demonstrated that MET improved the bladder dysfuntion associated with diabetes (31). On the other hand, bladder weights decreased after 30 days of ovariectomy. There was no statistically significant change in bladder wet weight in OVX rats after MET treatment. In the relaxation response, no difference was found between the OVX group and MET treated groups. This is supported by studies that show no effect on ovariectomy β adrenergic receptor expression in the bladder. Noradrenaline released from sympathetic nerve fibers causes tissue relaxation by activating β2 and/or β3-adrenoceptors in the bladder and contributes to bladder filling (32).

In concluson, MET treatment prevented detrusor muscle contractile dysfunction in OVX rats, suggesting that MET may have a therapeutic strategy for the treatment of menopause-related bladder dysfunction in women. The protective effect of metformin treatment on detrusor muscle dysfunction associated with menopause was demonstrated for the first time in this study. The results of the study suggest that metformin may provide therapeutic benefit for urologic complications related to menopause. A limitation of this study is that urodynamic parameters such as maximum voiding pressure and non-voiding contractions were not measured.

Further studies are needed for the therapeutic potential of MET in detrusor dysfunction induced by menopause.

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None.

Author Contributions

Hale Sayan Özaçmak and **İnci Turan** planned the study, **Meryem Ergenc** and **Salih Erdem** performed drug treatment and **Hale Sayan Özaçmak**, **İnci Turan,** and **Meryem Ergenc** and **Salih Erdem** carried out in vitro experiment, **Hale Sayan Özaçmak** and **İnci Turan** wrote and revised the manuscript.

Conflicts of Interest

The authors report no conflicts of interest.

Financial Disclosure

There are no financial support.

Ethical Approval

All experimental procedures were approved by the Zonguldak Bulent Ecevit University Animal Care and Use Ethical Committee (ethical approve number:2020-04-06/02).

Peer Review Process

Extremely peer-reviewed and accepted.

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