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RESEARCH ARTICLE

Effect of Trimebutine Maleate on Acetylcholine, Potassium chloride and Adenosine triphosphate Induced Contractions of Rat Detrusor Smooth Muscle

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SUMMARY

Overactive bladder (OAB) is a common clinical syndrome with a high prevalence in geriatric population. The main symptoms of OAB such as urgency, frequency and urge incontinence result from detrusor overactivity (DO) , that is due to involuntary contractions of the detrusor smooth muscle (DSM) in the filling phase of micturition. DSM contractility is mainly regulated by cholinergic and nonadrenergic, noncholinergic (NANC) mechanisms via multiple receptors. Acetylcholine (ACh)- induced contractions mediated by M_3 muscarinic receptors, adenosine triphosphate (ATP) –induced contractions mediated purinergic ion channel (P2X) receptors and Ca^{+2} influx are the fundamental contractile mechanisms of DSM, thus dysregulation of these contractile pathways cause DO and OAB.

Trimebutine maleate (TMB), a modulator of Ca^{+2} and K^{+} channels activity, has been widely prescribed to treat functional gastrointestinal disorders. However, its activity on bladder has not been investigated. To investigate the effects of TMB on rat DSM contractions induced by ACh, by potassium chloride (KCl) and by ATP; we performed in vitro organ bath studies on rat DSM strips. We reported that TMB pretreatment inhibited DSM contractions induced by ACh, KCl, ATP. Our findings suggest that TMB may be a potent inhibitor of DO and an effective drug for OAB.

Key Words: *Detrusor smooth muscle, overactive bladder, trimebutine maleate, in vitro organ bath, detrusor contractility.*

Trimebutin maleatın asetilkolin, potasyum klorür ve adenozin trifosfatla indüklenen sıçan detrüsör düz kas kontraksiyonları üzerine etkisi

ÖZET

Aşırı aktif mesane (AAM) , yaşlı popülasyonda prevalansı yüksek olan ve toplumda yaygın olarak görülen klinik bir sendromdur. AAM'nin temel semptomları olan sıkışma hissi , sık idrara çıkma ve sıkışma inkontinansı miksiyonun dolum fazında detrüsör aşırı aktivitesi olarak adlandırılan istemsiz detrüsör kası kasılmaları sonucunda oluşur. Detrüsör kası kontraksiyonları esas olarak adrenerjik, kolinerjik ve nonadrenerjik/non-kolinerjik (NANK) mekanizmalar tarafından yönetilir. M_3 reseptörleri aracılığıyla asetilkolinin (ACh), Pürinerjik P2X iyon kanalı reseptörleri aracılığıyla adenozin trifosfatın (ATP) ve hücre içerisine Ca^{+2} girişinin indüklediği kontraksiyonlar, mesanenin temel kontraksiyon mekanizmalarını oluşturmaktadır. Detrüsör aşırı aktivitesi ve AAM patofizyolojisinde kontraktil uyaranlara karşı aşırı cevap oluşumu yer alır. Ca^{+2} ve K^{+} kanallarının aktivitelerini modüle eden Trimebutin maleat (TMB), gastrointestinal sistemin hipo ve hiper motilite durumlarının tedavisinde yaygın olarak kullanılmaktadır. Literatürde TMB'nin sıçan detrüsör kası kontraktilitesi üzerine etkisini araştıran bir çalışmaya rastlanmamıştır. Bu çalışmada, TMB'nin sıçan detrösür düz kas (DDK) preparatlarında ACh, KCl ve ATP ile indüklenen kasılmalar üzerindeki etkisi ve buna bağlı olarak TMB'nin detrüsor aşırı aktivitesi ve AAM tedavisinde potansiyel terapötik etkisi in vitro olarak araştırılmıştır. Çalışma sonucunda, TMB inkübasyonunun sıçan DDK preparatlarında ACh, KCl ve ATP ile indüklenen kasılmaları inhibe ettiği gösterilmiştir. Çalışmamızın sonuçları, TMB'nin detrüsör aşırı aktivitesinin tedavisinde etkili bir ilaç olabileceğini önermektedir.

Anahtar kelimeler: *Aşırı aktif mesane, detrüsör , trimebutin maleat, in vitro organ banyosu, detrüsör kontraktilitesi*

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INTRODUCTION

Bladder contraction is mediated by both cholinergic and nonadrenergic, noncholinergic (NANC) mechanisms to coordinate the storage and voiding phases of bladder function. The smooth muscle of the bladder –the detrusor - has ability to contract and to relax and determines the bladder function during filling and voding phase of micturition (1). In many species, acetylcholine (ACh) is main neurotransmitter involved in detrusor smooth muscle (DSM) contractility via muscarinic (M) receptors and the contractile response to ACh is mediated predominantly by M_3 receptors . Ca^{+2} influx via voltage- activated Ca^{+2} channels is another mechanism to trigger detrusor contraction (2,3). It has been reported that L-type Ca^{+2} channel blocking agents, such as nifedipine, can inhibit the contractile force induced by Ca^{+2} influx in DSM strips (4). ATP has been also shown to involve in purinoceptor-mediated detrusor contractility. A number of recent studies have reported that multiple purinergic receptor subtypes are expressed in the bladder and ATP can cause both contraction or relaxation of DSM depending on subtype of receptors activated (1).

Overactive bladder (OAB) is a common condition which results from involuntary contractions of DSM termed as detrusor overactivity (DO) during the filling phase of micturition. Its prevalence increases with age and has a significant impact on the quality of life due to frequency, urgency and urge incontinence. It has been reported that excessive activity of contractile mechanisms of DSM leads to DO and OAB. Current pharmacotherapy of OAB and DO involves antimuscarinic drugs that have limited efficacy and number of side effects (5).

Timebutine maleate (TMB) has been used as an effective drug in the treatment of functional gastrointestinal disorders, however mechanism of its action remains unclear (6,7). TMB has local anesthetic, antimuscarinic and weak mu opioid agonist effects. It has also been shown to be able to regulate smooth muscle contractility of gastrointestinal tract via inhibiting L-type Ca^{+2} channels, Ca^{+2}-activated K^+ channels (8). However, the effects of TMB on DSM contractility is unclear. Therefore, in the present study we aimed to investigate the effects of TMB on rat DSM contractions induced by ACh, KCl and ATP as a potential future therapeutic for the treatment of OAB and DO.

MATERIALS AND METHODS

Preparation of DSM strips

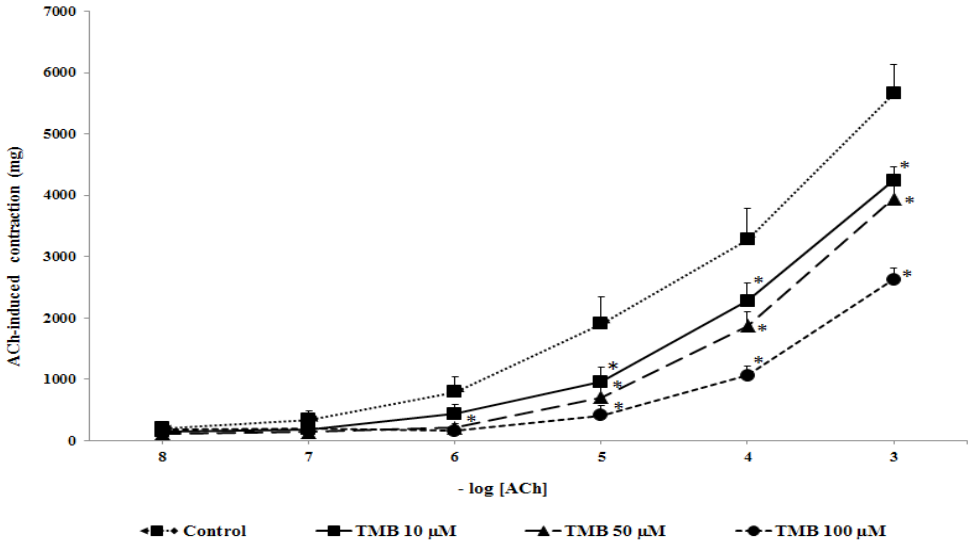
Female Spraque-Dawley rats (n= 15) weighing about 350-400 g were used in this study. Under anesthesia with ketamine (80 mg/kg;i.p) /xylazine (15 mg/kg;i.p), bladder was surgically removed and placed in cold Tyrode's solution (NaCI, 139.9 mM; KCI, 2.68 mM; $CaCl_2$, 1.8 mM; $MgCl_2$,1.05 mM; $NaHC0_3$, 11.9 mM; NaH_2P0_4, 0.42 mM and glucose, 5.55 mM). A longitudinal DSM strip (5–6 mm long and 2–3 mm wide) was prepared. Tissue strip was then placed in water-jacketed organ baths containing Tyrode solution at 37°C and bubbled with 95% O_2 and 5% CO_2 throughout the experiments. To measure tissue contractility, one end of the strip was attached to an isometric force transducer (MAY FDT10A) connected to data acquisition and recording system (BIOPAC MP 100). All procedures were approved by the local ethics committee of Karadeniz Technical University.

Contractility studies

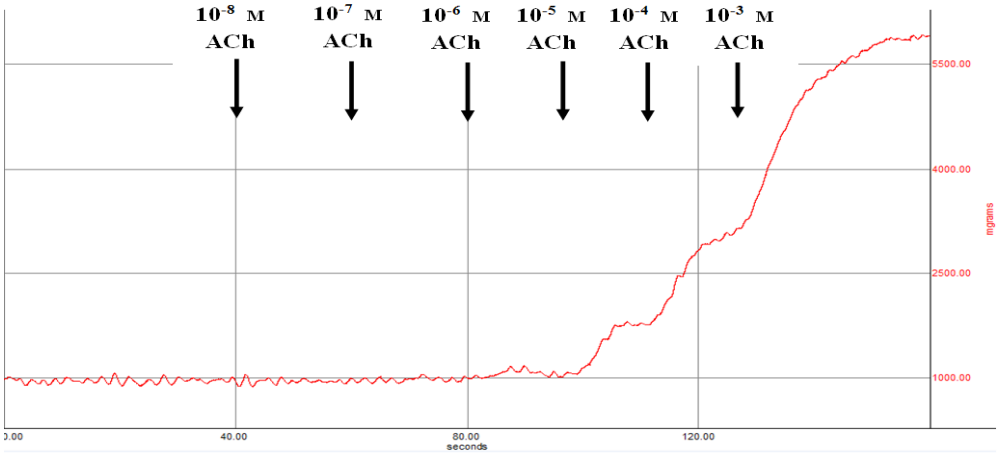
The strips were placed under 1 g resting tension and allowed to equilibrate for 60 min, with while replacing the bath solution every 20 min. After the equilibration period, to test the effect of TMB pretreatment on cholinergic DSM contractions, cumulative concentration-response to ACh (10^{-8} M - 10^{-3} M) was obtained in the absence (control) and in the presence of TMB (10, 50 and 100μM, 20 min) (n=5) . To test the effect of TMB pretreatment on KCl-induced contractions of DSM, contractile response to KCl (60 mM) was recorded in the absence (control) and in the presence of TMB (10, 50 and 100μM, 20 min) (n=5). To test the effect of TMB pretreatment on ATP (10^{-4} M) -induced contractions, contractile response to ATP(10^{-4} M) was obtained in the absence (control) and in the presence of TMB (10, 50 and 100μM, 20 min) (n=5). The contractility response were expressed as mg contraction or percentage (%) of control.

Statistical Analyses

Data were expressed as the mean ± S.E.M and analyzed by the one way ANOVA (Statistical Package for the Social Sciences, version 13.0, SSPS Inc, Chicago, IL, USA). $p<0.05$ was considered significant.


ACh-induced contraction (mg)
7000
6000
5000
4000
3000
2000
1000
0
8
7
6
5
4
3
- log [ACh]
Control
TMB 10 µM
TMB 50 µM
TMB 100 µM

A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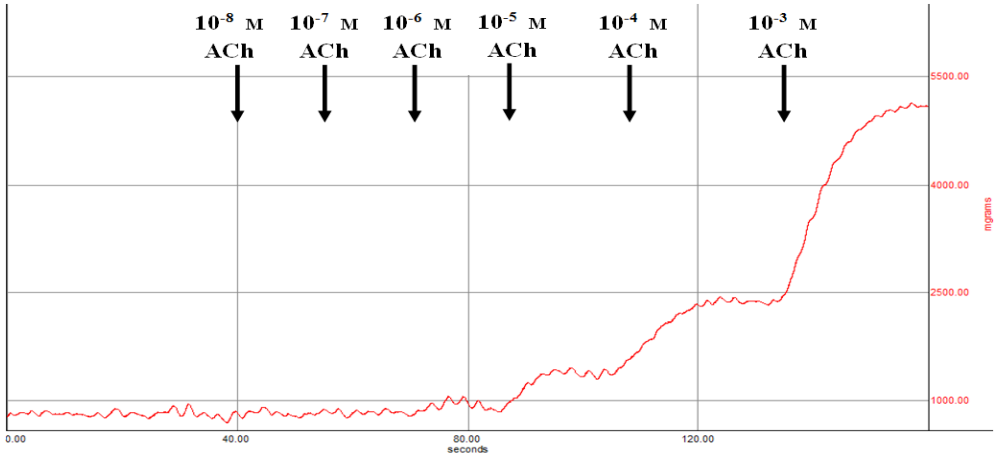
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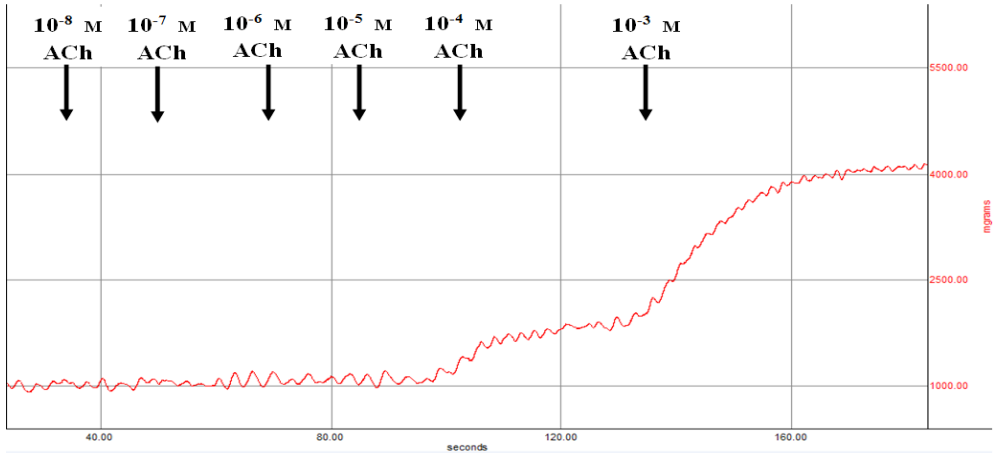

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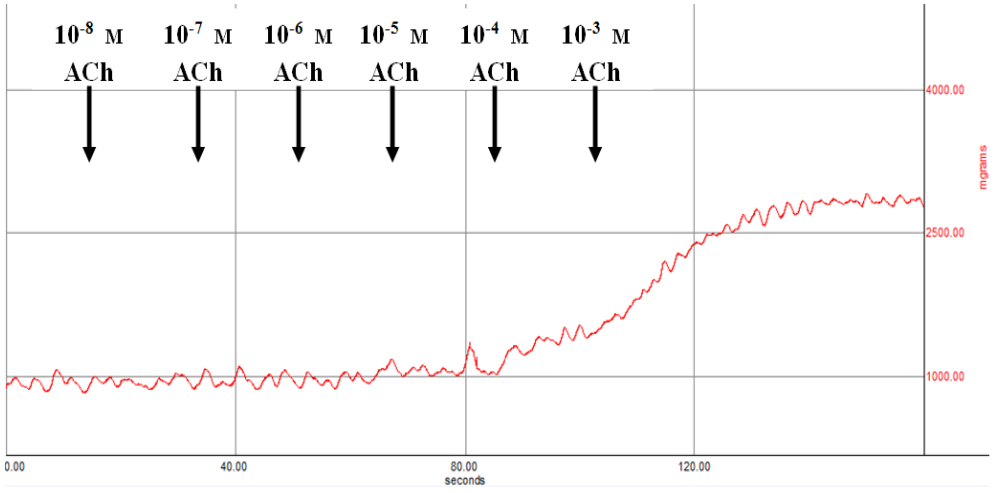

E

Figure 1. Effect of TMB incubation on ACh cumulative concentration-response(10^{-8} M - 10^{-3} M) . Summary data (**A**) and representative tracing of ACh before (**B**) and after TMB incubation (10 µM, C; 50 µM, D; 100 µM, E).*$p< 0.05$ vs control. The data were expressed as the mean ± S.E.M.

RESULTS

Effect of TMB pretreatment on ACh-induced contractions

ACh (10^{-8} M-10^{-3} M) -induced concentration dependent increase in DSM contractions. Pretreatment of DSM with TMB did not change baseline tension. However, ACh (10^{-8} M–10^{-3} M) induced contractions were significantly decreased in the presence of TMB compared with the response in the absence of TMB.

(Fig. 1A,1B, 1C, 1D and 1E).

Effect of TMB pretreatment on KCl-induced contractions

KCl (60 mM) -induced DSM contractions, which was significantly reduced by prior incubation with TMB (10, 50 and 100µM) compared with the response of the control (33%, 77% and 93%, respectively; Fig. 2A, 2B).

Effect of TMB pretreatment on ATP-induced contractions

ATP (10^{-8} M) -induced monophasic contraction in DSM strips. Incubation with 10 µM TMB did not

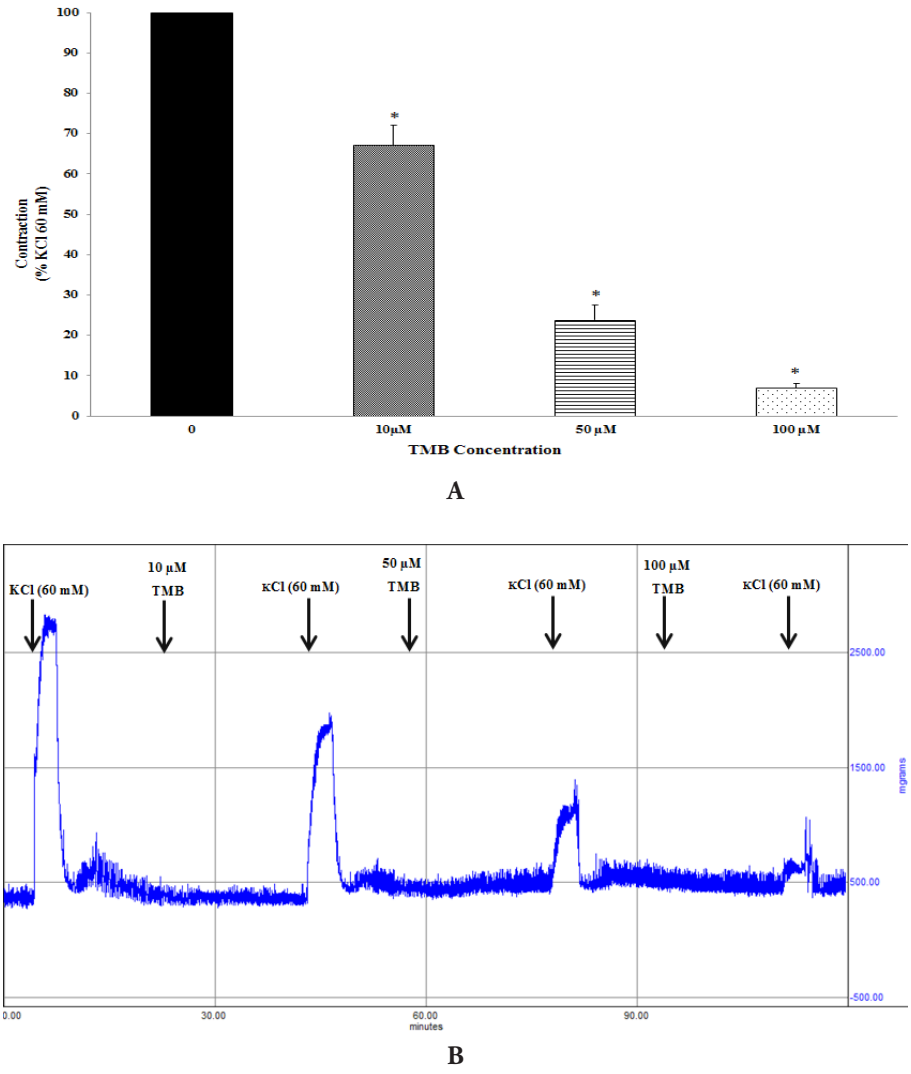

Figure 2. % KCl (60 mM) contraction in DSM strips when alone and pretreated with TMB. Summary data (A) and representative tracing of KCl-induced contractions before and after TMB (B) *p< 0.05 vs control. The data were expressed as the mean ± S.E.M.

change ATP-induced contractions when compared with the control. However, incubation with 50 and 100 µM of TMB caused ATP-induced relaxation (Fig. 3). Our preliminary studies with verapamil (10^{-6} M) pretreatment showed that verapamil reversed ATP-induced contractions. (Fig. 4) (n=2).

DISCUSSION

In the present study, we investigated the effect of TMB pretreatment on different contractile mechanisms in rat DSM and its possible mechanism of action mediating this effect. Our findings indicate that TMB pretreatment causes inhibition on ACh and KCl- induced contractions at all concentrations tested. ATP caused relaxation in DSM strips incubated with 50 µM and 100 µM TMB.

ACh is the main contractile transmitter involved in DSM contractility in many species. M_3 receptors are mediating the main part of contraction induced by ACh. Muscarinic agonist-induced contractions are believed to be mediated by non-selective cation channels and Rho-kinase activation (1).We observed a significant decrease in cholinergic contractions of DSM strips pretreated with TMB when compared to the contractile response of the control, indicating its antimuscarinic effect. Similarly, Long et al. showed that TMB diminished ACh-induced contractions in mouse colonic muscle strips (7) .

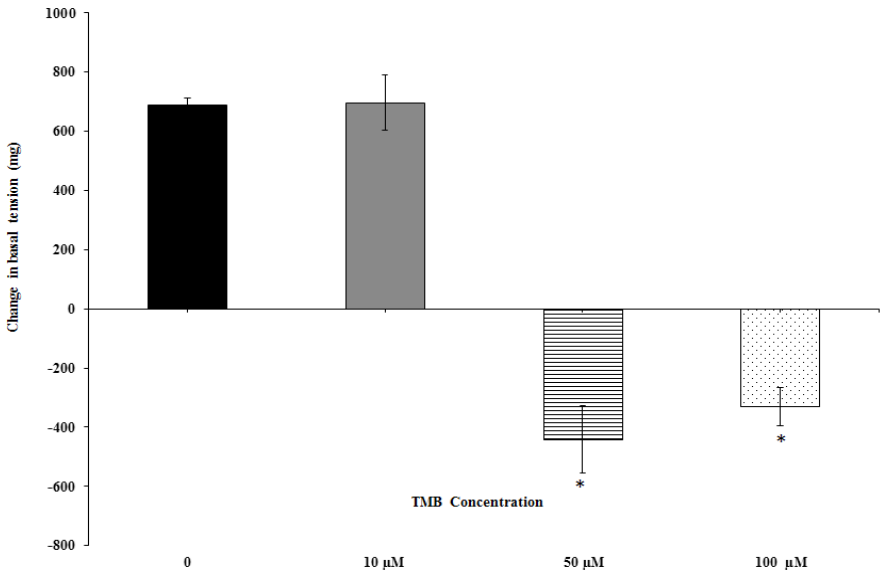

Figure 3. ATP (10^{-4} M)-induced tension of DSM strips pretreated with TMB.*p< 0.05 vs control. The data were expressed as the mean ± S.E.M.

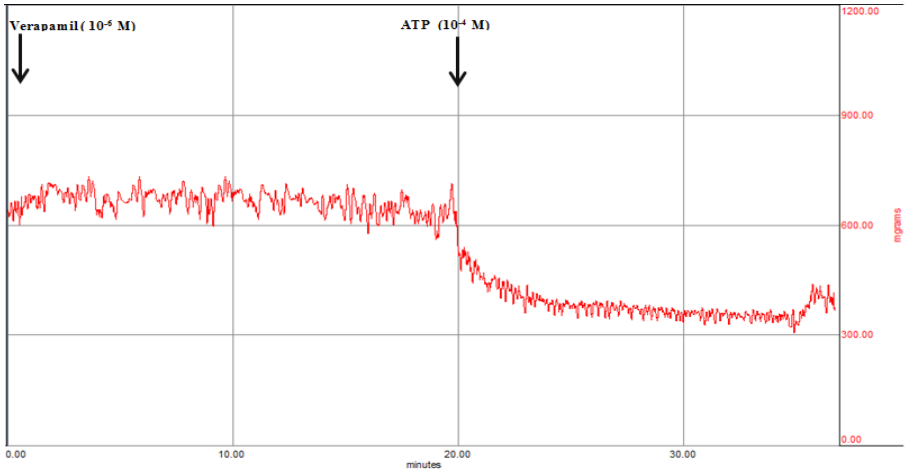

Figure 4. Representative tracing of ATP-induced relaxation on DSM strips pretreated with Verapamil (10^{-6} M).

Ca^{+2} influx predominantly through L-type Ca^{+2} channels is another mechanism involved in DSM contraction (1). We found that TMB pretreatment also decreased KCl-induced contractions via Ca^{+2} influx in DSM strips, indicating its L-type Ca^{+2} channel blocking effect. In the same line with our results, Tan et al. reported that TMB diminished L-type Ca^{+2} current and spontaneous contractions of colonic longitudinal muscle of guinea pigs (8).

ATP is an important regulator of bladder contractility. ATP binds to purinergic P_2 receptors that are divided into P2X and P2Y families. Whereas P2X ion channel receptors are mediating contraction, P2Y G protein-coupled receptors are leading to relaxation in urinary bladder (9). It has been well established that ATP-induced contraction mediated by P2X receptors is dependent on membrane depolarization and extracellular Ca^{+2} influx through L-type Ca^{+2} channels (1) . Bhat et al. showed that diltiazem and verapamil, which are Ca^{+2} channel blockers, significantly inhibited ATP-induced contraction in rat DSM (10). Burnstock et al. also reported that nifedipin, Ca^{+2} channel blocker, inhibited α,β-MetATP-induced contactions in rat DSM. They also demonstrated Ca^{+2} channel open-

er Bay K 8644 potentiated purinergic contractions of DSM (11). In the present study, we demostrated that ATP (10^{-4} M) caused relaxation in DSM strips incubated with higher concentration of TMB (50 and 100 μM). It is known that TMB can act as Ca^{+2} channel blocker at 30-300 μM (6). To determine whether Ca^{+2} channel antagonism underly the relaxing effect of ATP in TMB pretreated DSM strips, we evaluated the effect of verapamil, Ca^{+2} channel blocker, incubation on ATP-induced contractile response. Unlike the results of previous studies, our preliminary studies showed that ATP caused a relaxation in DSM strips incubated with verapamil (10^{-6} M) (Fig. 4). Therefore, the effect of TMB pretreatment on ATP-induced contractions of DSM might be due to its Ca^{+2} channel blocking effect. However, more studies are needed to clarify the mechanism of ATP-induced relaxation on DSM strips incubated with TMB (50 and 100 μM).

CONCLUSION

To our knowledge, this is the first study demonstrating the effect of TMB on rat detrusor contractility. Our data showed that TMB pretreatment inhibits ACh, KCl, ATP-induced contractions in rat DSM strips. This effect appears to be related with its antimuscarinic and Ca^{+2} channel blocking action. Antimuscarinic agents such as oxybutynin, tolterodine, darifenacin are the first-line drugs for the treatment of OAB and DO. However, these drugs have poor patient compliance and limited long term efficacy (5) .Therefore, new drugs are required with improved efficacy and tolerability. Our results suggest that TMB has a potantial to be an effective drug for the treatment of OAB and DO.

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