Review

Effects of Distigmine on the Mechanical Activity of Urinary Bladder Smooth Muscle

Keisuke Obara* and Yoshio Tanaka

Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, Toho University; 2–2–1 Miyama, Funabashi, Chiba 274–8510, Japan.
Received February 28, 2019; accepted April 24, 2019

Distigmine bromide (distigmine) is a reversible carbamate cholinesterase (ChE) inhibitor. Its principle clinical application is in the treatment of myasthenia gravis. Distigmine is also used as a remedy for dysuria and glaucoma. Its effectiveness in the management of dysuria has been demonstrated in several clinical reports. Distigmine may improve (enhance) urinary bladder smooth muscle (UBSM) contraction during micturition by inhibiting acetylcholine (ACh) decomposition. However, the pharmacological effects of distigmine on UBSM have not been adequately studied so far. In this review article, we summarize the reported effects of distigmine on the contractile responses elicited by exogenous and endogenous ACh in isolated UBSM preparations. We also discuss the effects of distigmine on the UBSM basal tone and the contractile response of UBSM to ATP, which is co-released with ACh from parasympathetic nerve terminals.

Key words distigmine bromide; urinary bladder smooth muscle; cholinesterase inhibitor; acetylcholine; adenosine triphosphate

1. INTRODUCTION

Distigmine bromide (distigmine) is a carbamate cholinesterase (ChE) inhibitor first synthesized by Schmid in the 1950s (Fig. 1). It and other carbamate ChE inhibitors have been used to treat myasthenia gravis in Asia, the Middle East, and Europe.2–5 Distigmine has also been used to treat glaucoma in Japan.6–8 Distigmine has also been applied as a remedy for dysuria in Japan since its release there in 1968. It was reported that distigmine has a therapeutic effect on neurogenically underactive bladder caused by surgery, spinal cord injury, and chronic diseases such as diabetes.9–21 It is also effective against drug-induced dysuria22,23 and benign prostatic hyperplasia (BPH)-induced dysuria.24–26 Distigmine was reported to be clinically effective against both underactive bladder and dysuria. In recent years, distigmine efficacy was validated with various animal disease models.27–29

The therapeutic effect of distigmine in the management of urinary disorders is explained by its ChE inhibition. This mechanism enhances the contractile force of the urinary bladder smooth muscle (UBSM) by increasing the concentration of acetylcholine (ACh) in the synaptic cleft between the parasympathetic nerve terminal and the UBSM. However, the pharmacological effects of distigmine on the UBSM have seldom been investigated despite the fact that numerous clinical reports have demonstrated its efficacy in dysuria treatment. In this review article, we present a synopsis of studies in which the effects of distigmine on the contractile responses elicited by exogenous and endogenous ACh were examined in isolated UBSM preparations. We also discuss the effects of distigmine on the UBSM basal tone and the contractile response of UBSM to the neurotransmitter ATP, which is co-released with ACh from the parasympathetic nerve terminals.

2. EFFECTS OF DISTIGMINE ON UBSM CONTRACTILE RESPONSES ELICITED BY EXOGENOUS CONSTRICITORS

During urination, ACh and ATP are released from the parasympathetic nerve ending in the UB in response to nerve excitation.30–36 Distigmine inhibits acetylcholinesterase (AChE) activity in the UB.37,38 Therefore, it is presumed that distigmine enhances the contractile force of the UBSM by increasing ACh concentration in the synaptic cleft between the parasympathetic nerve ending and the UBSM. To test this assumption, we investigated the effects of distigmine on ACh-induced contractions in guinea pig UBSM. As shown in Fig. 2A, distigmine (3 × 10−8–3 × 10−6 M) enhanced the ACh-induced guinea pig UB contraction.40–42 These results, then, support the presumption that distigmine enhances the contractile force of UBSM by increasing ACh.

A study using the balloon method reported that distigmine potentiated the increase in guinea pig intravesical pressure induced by intravenous ACh administration.39 The duration of the enhancement of ACh-induced guinea pig UB contraction by distigmine was commensurate with its inhibitory effect on ChE in the UB.42 These results, then, support the presumption that distigmine enhances the contractile force of UBSM by increasing ACh.

ATP is also a parasympathetic UBSM constrictor. Thus, we also examined the effect of distigmine on ATP-induced guinea

Fig. 1. Structure of Distigmine Bromide (Distigmine)
pig UBSM contractile response. However, distigmine (10^{-6}M) had no effect on the ATP concentration-response curves in guinea pig UBSM (Fig. 2B).

3. EFFECTS OF DISTIGMINE ON UBSM CONTRACTILE RESPONSES ELICITED BY ENDOGENOUS PARASYMPATHETIC NEUROTRANSMITTERS

In human UBSM, distigmine may enhance contractile responses to parasympathetic nerve excitation induced by electrical field stimulation (EFS). However, it is unknown which endogenous parasympathetic neurotransmitters cause this potentiation. Guinea pig UBSM contractile responses to EFS consisted of a contractile component sensitive to the muscarinic receptor antagonist atropine and another affected by the purinergic receptor-desensitizing agent α,β-methylene ATP (α,β-mATP). The application concentrations of these agents were 10^{-6} and 10^{-7}M, respectively (Fig. 3A). Therefore, these contractions were caused by both ACh (the atropine-sensitive component) and ATP (the α,β-mATP-sensitive component).

To confirm that distigmine augments the ACh-induced contractile component, we examined its effects on contraction generated in the presence of α,β-mATP (10^{-5}M). Distigmine (10^{-6}M) substantially enhanced the contractile component and subsequent atropine (10^{-6}M) administration almost completely suppressed it (Fig. 3B). Therefore, distigmine clearly augmented the ACh-mediated contractile component.

We also examined the effects of distigmine on the EFS-induced contractile component generated in the presence of atropine to verify that distigmine does not influence ATP-induced contraction. Distigmine (10^{-6}M) did not significantly affect the contractile component generated in the presence of atropine (10^{-6}M) (p > 0.05 for atropine (10^{-6}M) vs. atropine (10^{-6}M) + distigmine (10^{-6}M)). In fact, the contractile component generated in the presence of atropine was mediated by ATP because it was significantly suppressed by α,β-mATP (10^{-4}M) (p < 0.05 for atropine (10^{-6}M) vs. atropine (10^{-6}M) + distigmine (10^{-6}M) + α,β-mATP (10^{-4}M); p < 0.01 for atropine (10^{-6}M) + distigmine (10^{-6}M) vs. atropine (10^{-6}M) + distigmine (10^{-6}M) + α,β-mATP (10^{-4}M)) (Fig. 3C). These results suggest that distigmine enhances UB contraction during urination by potentiating UBSM contraction mediated by the release of endogenous ACh from the parasympathetic nerve terminals without affecting ATP-mediated contraction. Similar results were obtained for mouse bladder.

4. EFFECTS OF DISTIGMINE ON THE BASAL TONE OF UBSM

Very close observation disclosed that distigmine marginally increased the basal tone of guinea pig UBSM (Fig. 4). Distigmine also very slightly increased basal tone in human UBSM. In contrast, the carbamate ChE inhibitors neostigmine and pyridostigmine strongly enhanced the basal tone of guinea pig UBSM. This discrepancy may be resolved by the fact that whereas neostigmine and pyridostigmine are exclusively cholinergic, distigmine may also have a paradoxical anticholinergic effect. In rat bladder, distigmine inhibited the binding of [3H]N-methyl scopolamine to the muscarinic receptors. Its Kᵢ value was 1.33 ± 0.11 μM whereas that for neostigmine was >50 μM. Therefore, the latter should have no effect on the muscarinic receptors. Our mechanistic study demonstrated that distigmine (3 × 10^{-5}M) suppressed betahanechol-induced contractile responses in guinea pig UBSM and urethral smooth muscle. Betahanechol is a synthetic choline ester which is not decomposed by ChE.

ChE inhibitors may increase UBSM basal tone by stimulating the release of ACh from parasympathetic nerve endings. This mechanism was inferred from the effects of ChE inhibitors on nicotine receptors. Distigmine and neostigmine inhibit the binding of [3H]epibatidine to nicotine receptors in rat cerebral cortex. The Kᵢ values were 22.9 ± 3.3 μM for distigmine and 18.8 ± 3.3 μM for neostigmine. We investigated whether neostigmine mediated the increase in UBSM basal tone via ACh derived from parasympathetic nerves. This effect was not significantly affected by tetrodotoxin (TTX; 10^{-6}M) (p > 0.05 for neostigmine (3 × 10^{-5}M) vs. neostigmine (3 × 10^{-5}M) + TTX (10^{-6}M)) (our unpublished observation). Therefore, ACh derived from the parasympathetic nerves did not significantly contribute to the neostigmine-induced increase in UBSM basal tone. Non-neurogenic ACh was continuously detected in UB tissue irrespective of the presence of urothelium. However, the contribution of the urothelium was greater than that of the UBSM. Therefore, neurogenic ACh
may not be involved in this process. Rather, non-neurogenic components may participate in it. It is possible, then, that ACh is continuously supplied to UB tissue without parasympathetic nerve excitation. The inhibition of ChE by neostigmine and distigmine may increase UBSM basal tone by activating the muscarinic receptors. However, non-neurogenic ACh might be

---

Fig. 3. Electrical Field Stimulation (EFS)-Induced Contractile Responses of Guinea Pig Urinary Bladder (UB) Preparation

A: Representative trace showing EFS-induced contractions and the effects of atropine (10^{-6} M), α,β-methylene ATP (α,β-mATP) (10^{-4} M), and tetrodotoxin (TTX) (3 × 10^{-7} M). All drugs were applied cumulatively in the indicated order. B: Representative traces showing the potentiating effects of distigmine (10^{-6} M) and the inhibitory effects of atropine (10^{-6} M) on EFS-evoked contractions in the presence of α,β-mATP (10^{-4} M). C: Representative traces showing the effects of distigmine (10^{-6} M) and α,β-mATP (10^{-4} M) on EFS-evoked contractions in the presence of atropine (10^{-6} M). All experiments were conducted in the presence of phentolamine (10^{-6} M), propranolol (10^{-6} M), and indomethacin (3 × 10^{-6} M) to block the potential effects of endogenous noradrenaline and prostaglandins. Data were modified with the permission of the authors of Eur. J. Pharmacol., 809, 209–214 (2017).44)

Fig. 4. Direct Contractile Effects of Distigmine (A) and Neostigmine (B) on Isolated Guinea Pig Urinary Bladder Preparations

The contractile effects of distigmine and neostigmine were evaluated as the transient maximum responses appearing usually within 5 min after administration (Max.) and the tension levels at 30 and 60 min after administration. Distigmine and neostigmine were applied to the bath solution as a single administration. Ordinate: % contractions induced by 3 × 10^{-4} ACh. Abscissa: negative logarithms of the distigmine concentrations (M). Data are collected from n = 2–5 experiments per distigmine concentration and n = 2–7 experiments per neostigmine concentration and reproduced with the permission of the authors of Ōyō Yakuri/Pharmacometrics, 75, 85–96 (2008).40)
suppressed by the anticholinergic action of distigmine so that the basal tone only slightly increases.

5. EFFECTS OF DISTIGMINE ON INTRACELLULAR UBSM MECHANISMS

It was reported that the muscarinic M₃ receptors are expressed to a greater extent than the M₁ receptors in mammalian UBSM.⁴⁹ However, it is mainly the M₁ receptor rather than the M₃ receptor which triggers ACh-induced UBSM contraction in normal UB.⁴⁹ Stimulation of the muscarinic M₁ receptor in UBSM activates phospholipase C (PLC) expected by the typical intracellular mechanisms associated with Gq protein-coupled receptors.⁵⁹ However, ACh-induced, M₃ receptor-mediated UBSM contraction may be unaffected by PLC inhibition. It could, however, be associated with the activation of Rho kinase and/or voltage-dependent Ca²⁺ channels.⁴⁹ It was recently proposed that the TRPC4 channel significantly contributes to the mechanism underlying voltage-dependent Ca²⁺ channel activation.⁵⁰ Therefore, distigmine-induced enhancement of ACh-mediated UBSM contraction may be triggered by Rho kinase and/or TRPC4 channel activation.

On the other hand, ATP-induced UBSM contraction is mediated mainly by ionotropic P₂X₁ receptors.⁵¹ P₂X₁ receptors are ligand-gated cation channels. When they are stimulated by ATP, they enhance Ca²⁺ influx and sensitization of the contractile apparatus to Ca²⁺.⁵¹ However, it is unlikely that distigmine affects ionotropic P₂X₁ receptor-coupled intracellular pathways since it has no apparent influence on ATP-induced contraction in the UBSM.

Indirect or direct muscarinic receptor stimulators such as distigmine and bethanechol have been administered for many years to treat underactive bladder and remain the agents of choice for the management of this symptom. In recent years, however, ONO-8055, an agonist of the prostanooid EP₂ and EP₃ receptors, was reported to be effective in a radical hysterecomy-induced underactive monkey bladder model.²⁷ Thus, prostanoid receptor-related drugs may prove to be efficacious therapeutic agents for the management of underactive bladder.

6. CONCLUSION

The UBSM contracts in response to parasympathetic nerve stimulation mediated by ACh and ATP. Distigmine augments the contractile response of UBSM by enhancing the neurogenic ACh-mediated contractile component resulting from the inhibition of ACh degradation (Fig. 5). Suppression of non-neurogenic ACh degradation by ChE inhibitors may increase UBSM basal tone. However, a possible anticholinergic action of distigmine counteracts this effect (Fig. 5). Thus, this drug has only a slight net effect on the apparent UBSM basal tone. Therapeutic agents for underactive bladder are limited. Therefore, elucidation of the pharmacological effects and molecular mechanisms of distigmine could facilitate the development of new drugs for the management of lower urinary tract dysfunction.

Conflict of Interest

The authors declare no conflict of interest.

REFERENCES
