Regular Article

Noradrenaline-Induced Relaxation of Urinary Bladder Smooth Muscle Is Primarily Triggered through the \( \beta_3 \)-Adrenoceptor in Rats

Keisuke Obara,\(^{a}\) Serena Suzuki,\(^{a}\) Hiroko Shibata,\(^{a}\) Naoki Yoneyama,\(^{a}\) Shoko Hamamatsu,\(^{a}\) Fumiko Yamaki,\(^{a}\) Koji Higai,\(^{b}\) and Yoshio Tanaka*\(^{a,}\)\(^{\ast}\)

\(^{a}\) Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, Toho University; 2–2–1 Miyama, Funabashi, Chiba 274–8510, Japan; and \(^{b}\) Laboratory of Medical Biochemistry, Faculty of Pharmaceutical Sciences, Toho University; 2–2–1 Miyama, Funabashi, Chiba 274–8510, Japan.

Received November 18, 2018; accepted January 25, 2019

\( \beta \)-Adrenoceptors are subclassified into 3 subtypes (\( \beta_1 - \beta_3 \)). Among these, \( \beta_3 \)-adrenoceptors are present in various types of smooth muscle and are believed to play a role in relaxation responses of these muscles. \( \beta_3 \)-Adrenoceptors are also present in urinary bladder smooth muscle (UBSM), although their expression varies depending on the animal species. To date, there has been little information available about the endogenous ligand that stimulates \( \beta_3 \)-adrenoceptors to produce relaxation responses in UBSM. In this study, to determine whether noradrenaline is a ligand of UBSM \( \beta_3 \)-adrenoceptors, noradrenaline-induced relaxation was analyzed pharmacologically using rat UBSM. We also assessed whether noradrenaline metabolites were ligands in UBSM. In isolated rat urinary bladder tissues, mRNAs for \( \beta_1 \), \( \beta_2 \), and \( \beta_3 \)-adrenoceptors were detected using RT-PCR. In UBSM preparations contracted with methacholine (3 \( \times \) \( 10^{-5} \) M), noradrenaline-induced relaxation was not inhibited by the following antagonists: atenolol (10 \( \times \) \( 10^{-5} \) M; selective \( \beta_1 \)-adrenoceptor antagonist), ICI-118,551 (3 \( \times \) \( 10^{-5} \) M; selective \( \beta_2 \)-adrenoceptor antagonist), propranolol (10 \( \times \) \( 10^{-7} \) M; non-selective \( \beta \)-adrenoceptor antagonist), and bupranolol (10 \( \times \) \( 10^{-6} \) M; non-selective \( \beta \)-adrenoceptor antagonist). In the presence of propranolol (10 \( \times \) \( 10^{-6} \) M), noradrenaline-induced relaxation was competitively inhibited by bupranolol (3 \( \times \) \( 10^{-7} \) M) or SR59230A (10 \( \times \) \( 10^{-5} \) M; selective \( \beta_3 \)-adrenoceptor antagonist), with their p.A values calculated to be 6.64 and 7.27, respectively. None of the six noradrenaline metabolites produced significant relaxation of methacholine-contracted UBSM. These findings suggest that noradrenaline, but not its metabolites, is a ligand for \( \beta_3 \)-adrenoceptors to produce relaxation responses of UBSM in rats.

**Key words**  rat urinary bladder smooth muscle; noradrenaline; \( \beta \)-adrenoceptor

INTRODUCTION

The urinary bladder (UB) is an organ that stores urine and discharges it outside the body. These physiological functions are controlled by the relaxation and contraction responses of UB smooth muscle (UBSM), and both responses are affected strongly by autonomic nerves. In particular, urine discharge is associated with UBSM contraction. This contraction is principally triggered by acetylcholine that is released from parasympathetic nerve endings, the activity of which predominates in the micturition phase. In contrast, urine storage (retention) is strongly by autonomic nerves. In particular, urine discharge is mainly triggered by acetylcholine that is released from sympathetic nerve endings, the activity of which predominates in the UB filling phase.\(^{1-3}\) However, how sympathetic nerves contribute to the relaxation of UBSM and urine storage is not completely understood.

If noradrenaline is a key molecule to trigger UBSM relaxation, it is reasonable to postulate that it would target \( \beta \)-adrenoceptors in UBSM, as it does in other smooth muscles. \( \beta \)-Adrenoceptors are classified into 3 subtypes (\( \beta_1 - \beta_3 \))\(^{4,5}\) and UBSM subtype expression is species-dependent.\(^{51}\) In humans, the main \( \beta \)-adrenoceptor subtype in UBSM has been identified as \( \beta_3 \) at the mRNA level.\(^{5,6}\) In addition, a selective \( \beta_3 \)-adrenoceptor agonist, mirabegron, has been reported to induce UBSM relaxation and improve overactive bladder (OAB) symptoms by increasing bladder capacity.\(^{7-9}\) These findings suggest that \( \beta_3 \)-adrenoceptors play a significant role in the regulation of the UBSM relaxation response and, thus, urine storage. However, whether noradrenaline acts as an endogenous ligand for the \( \beta_3 \)-adrenoceptor to induce UBSM relaxation has not been convincingly established. This is because almost all pharmacological studies on UBSM \( \beta \)-adrenoceptors were designed using synthetic \( \beta \)-adrenoceptor agonists (isoprenaline or selective \( \beta_1 \)-adrenoceptor agonists), but not endogenous catecholamines such as noradrenaline.

The purpose of this study was to investigate whether noradrenaline is a ligand for the \( \beta_3 \)-adrenoceptor using rat UBSM tissue. This tissue expresses all subtypes of \( \beta \)-adrenoceptors\(^{10,11}\) allowing the determination of whether noradrenaline can stimulate \( \beta_1 \) and \( \beta_2 \) subtypes in addition to \( \beta_3 \). In this study, we also examined six metabolites of noradrenaline in order to verify whether they can induce UBSM relaxation via stimulation of \( \beta_3 \)-adrenoceptors.

MATERIALS AND METHODS

**Drugs** The following drugs were used: (\( \pm \))-atenolol, desipramine hydrochloride, 3,4-dihydroxymandelic acid (DOMA), \( \Delta_3 \)-3,4-dihydroxyphenylglycol (DHPG), 3,5-dinitrocatechol, \( \Delta_3 \)-4-hydroxy-3-methoxymandelic acid (VMA), 4-hydroxy-3-methoxyphenyl glycol (MHPG) hemipiperazinium salt, 4-hydroxy-3-methoxyphenylglycol sulfate (MHPG-S) potassium salt, indomethacin, ICI-118,551 hydrochloride, (\( \pm \))-isoproteorenol (ISO) hydrochloride, \( \Delta_3 \)-normetanephrine (NMN) hydrochloride, N-methyl-N-propargyl-3-(2,4-dichlo-
rophenoxy)propylamine (clorgiline) hydrochloride, DL-propranolol hydrochloride, SR 59230A (all from Sigma-Aldrich Co., St. Louis, MO, U.S.A.); (±)-phenolamine mesylate (Novartis Pharma, Basel, Switzerland); acetyl-β-methylcholine (methylcholine) chloride, (R)-(−)-norepinephrine (noradrenaline) hydrogen tartrate monohydrate (both from Wako Pure Chemical Industries, Ltd., Osaka, Japan); and (±)-bupranolol hydrochloride (Kaken Pharmaceutical Co., Ltd., Tokyo, Japan). All other chemicals were commercially available and reagent grade.

Atenolol was dissolved in 0.1 N hydrochloric acid (HCl) as a stock solution at 2 × 10⁻² M and diluted with distilled water. Indomethacin was dissolved in pure ethanol as a stock solution at 10⁻¹ M. 3,5-Dinitrocatechol was dissolved in dimethyl sulfoxide (DMSO) as a stock solution at 4 × 10⁻³ M and diluted with distilled water. SR 59230A was dissolved in DMSO as a stock solution at 2 × 10⁻² M and diluted with distilled water. All other drugs were prepared as aqueous stock solutions and diluted with distilled water.

Animals Male Wistar rats (8–10 weeks old; weight 165–265 g, Sankyo Labo Service Corporation, Tokyo, Japan) were housed under controlled conditions (21–22°C, relative air humidity 50 ± 5%, fixed 12 h light–dark cycle (08:00–20:00)) with food and water available ad libitum. This study was approved by the Toho University Animal Care and User Committee (approval number: 16-52-294, approved on May 16, 2016; approval number: 17-53-294, approved on May 17, 2017) and was conducted in accordance with the User’s Guideline to the Laboratory Animal Center of Faculty of Pharmaceutical Sciences, Toho University.

RT-PCR Analysis of β-Adrenoceptor Subtype mRNA Expression The rats were anaesthetized with isoflurane (inhalation) and euthanized by exsanguination from a carotid artery. The UB, atrium (both right and left atria), and ileum were immediately removed and placed in normal Tyrode’s solution of the following composition (mM): NaCl, 158.3; KCl, 4.0; CaCl₂, 2.0; MgCl₂, 1.05; NaH₂PO₄, 0.42; NaHCO₃, 10.0; and glucose, 5.6. All tissues were stripped of surrounding adipose tissue, connective tissue, and bladder trigone from the UB preparations were treated with methacholine (3 × 10⁻³ M). When the contraction reached steady-state, the preparation was relaxed by applying isoprenaline. This procedure was performed twice before starting the experiment (performed 3 times in total). All experiments were carried out in the presence of indomethacin (3 × 10⁻⁶ M) to prevent any possible effects of endogenous prostaglandins.

Evaluation of Effects of β-Adrenoceptor Antagonists on Noradrenaline-Induced Relaxation After conducting the preliminary procedures described in the previous section, the UB preparations were treated with methacholine (3 × 10⁻³ M). When the contraction was relaxed, the UB preparations were treated with the indicated β-adrenoceptor antagonists (atenolol (10⁻⁶ M), ICI-118,551 (3 × 10⁻⁶ M), propranolol (10⁻⁷–10⁻⁶ M), bupranolol (10⁻⁷–3 × 10⁻⁶ M), and SR 59230A (10⁻⁷–10⁻⁶ M). When bupranolol (3 × 10⁻⁷–3 × 10⁻⁶ M) or SR 59230A (10⁻⁷–10⁻⁶ M) was administered, the experiment was performed in the presence of propranolol (10⁻⁶ M) according to previous studies. This series of experiments was carried out in the presence of desipramine (3 × 10⁻⁷ M) as an uptake-1 inhibitor, NNM (10⁻⁶ M) as an uptake-2 inhibitor, and phenolamine (10⁻⁶ M) as a non-selective α-adrenoceptor antagonist to prevent possible effects of noradrenaline reuptake or α-adrenoceptors. These drugs were administered 20 min before methacholine.

Evaluation of Effects of Noradrenaline Metabolites on Methacholine-Induced Contraction After conducting the preliminary procedures described in “Preparation of UB Strips and Recording of Isotonic Tension Changes,” the UB
preparations were treated with methacholine (3 × 10^{-5} M). When the contraction reached a steady-state level, noradrenaline or the indicated noradrenaline metabolites (10^{-4} M each) was applied to the bath solution. Ten minutes after the administration, isoprenaline (10^{-4} M) was applied to confirm that the UB preparation was sufficiently relaxed.

This series of experiments was carried out in the presence of clorgiline (10^{-5} M) as a monoamine oxidase A (MAO A) inhibitor, 3,5-dinitrocatechol (2−yltransferase (COMT) inhibitor, and phentolamine (10^{-5} M) to prevent any possible effects of metabolism of noradrenaline or α-adrenoceptors. These drugs were administered 20 min before methacholine.

**Data Analysis** The extent of relaxation induced by noradrenaline and the six noradrenaline metabolites was calculated relative to the tone level before the application of 3 × 10^{-5} M methacholine (100% relaxation), and to the steady-state tone level prior to the application of each relaxant (0% relaxation).

The potencies of noradrenaline were calculated as pD_{2} (pEC_{50}) values (the negative logarithm of the effective agonist concentration producing a response that is 50% of the maximum response). The data were plotted as a function of noradrenaline concentration and fitted to the equation:

\[ E = E_{\text{max}} \times A^{n_{E}} / (E_{C_{50}}^{n_{E}} + A^{n_{E}}) \]

where \( E \) is the % relaxation at a given concentration, \( E_{\text{max}} \) is the maximum response, \( A \) is the noradrenaline concentration, \( n_{E} \) is the Hill coefficient, and \( E_{C_{50}} \) is the agonist concentration producing a 50% response. Curve-fitting was carried out using GraphPad Prism (Version 6.07; GraphPad Software, Inc., San Diego, CA, U.S.A.).

The β-adrenoceptor antagonist potencies are expressed as pA_{2} values, which were calculated according to the method originally reported by Arunlakshana and Schild.14

Data are expressed as means ± standard error of the mean (S.E.M.) or means with 95% confidence intervals (95% CIs) and \( n \) refers to the number of experiments. The significance of the differences between mean values was evaluated by two-way ANOVA or paired t-tests using GraphPad Prism. A \( p \)-value less than 0.05 was considered statistically significant.

**RESULTS**

**Expression of mRNAs for β-Adrenoceptor Subtypes in Rat UB Preparations** Figure 1 shows representative images of agarose gels for β1- , β2-, and β3-adrenoceptor PCR products in rat UB, atrium (both right and left atria) (A), and ileal longitudinal smooth muscle (I), with the expected PCR products of 337, 386, and 352 base pairs, respectively. In both the UB and ileal longitudinal smooth muscle, mRNAs for all 3 β-adrenoceptors (β1- , β2-, and β3-) were detected. In contrast, in the atrium, β1- and β3-adrenoceptor mRNAs were clearly detected, but that of β2-adrenoceptor was absent or barely detected. The PCR product for β-actin, as an internal standard, was detected in all 3 preparations; this had the expected size of 375 base pairs. No bands were observed in the absence of reverse transcription (RT(−)).

**Effects of Various Antagonists for β-Adrenoceptors on Noradrenaline-Induced Relaxation** Figure 2A shows the effects of repeated noradrenaline administration on its concentration-relaxation curves in rat UBSM. The response curves of noradrenaline did not change in both the first and second applications; this was evidenced by the lack of statistically significant differences in both pD_{2} (5.85 ± 0.08 for first and 5.83 ± 0.05 for second, \( n \) = 8 each, \( p > 0.05 \)) and \( E_{\text{max}} \) (52.4 ± 4.4% for first and 50.3 ± 3.0% for second, \( n \) = 8 each, \( p > 0.05 \)) values between the first and second applications.

Figures 2B–F show the effects of various types of β-adrenoceptor antagonists on noradrenaline-induced relaxation. The tested β-adrenoceptor antagonists were: atenolol (a selective β1-adrenoceptor antagonist, 10^{-6} M) (Fig. 2B), ICI-118,551 (a selective β3-adrenoceptor antagonist, 3 × 10^{-7} M) (Fig. 2C), propranolol (a nonselective β-adrenoceptor antagonist, 10^{-7} M, 10^{-6} M) (Figs. 2D, E, respectively), and bupranolol (a nonselective β-adrenoceptor antagonist, 10^{-7} M) (Fig. 2F). Noradrenaline-induced relaxation was not affected by atenolol, ICI-118,551, 10^{-7} M propranolol, or bupranolol (10^{-5} M). However, the noradrenaline-induced relaxation curve was shifted rightward by approximately 2-fold by 10^{-6} M propranolol (Fig. 2E).

**Effects of Bupranolol and SR 59230A on Noradrenaline-Induced Relaxation** Figure 3A shows the effects of repeated noradrenaline administration on its concentration-relaxation curves in rat UBSM in the presence of propranolol (10^{-6} M). The response curves of noradrenaline did not change in both the first and second applications; this was demonstrated by the absence of statistically significant differences in both pD_{2} (5.36 ± 0.04 for first and 5.32 ± 0.03 for second, \( n \) = 12 each, \( p > 0.05 \)) and \( E_{\text{max}} \) (45.6 ± 2.0% for first and 43.6 ± 1.9% for second, \( n \) = 12 each, \( p > 0.05 \)) values between the first and second applications.

Figures 3B–D show the effects of bupranolol on noradrenaline-induced relaxation in the presence of propranolol (10^{-6} M). The noradrenaline-induced relaxation was inhibited by bupranolol (3 × 10^{-7}–3 × 10^{-6} M) in a concentration-dependent manner, shifting the corresponding concentration-response curve rightward (Figs. 3B–D). Figure 3E shows the Schild plot of bupranolol against noradrenaline based on the results of Figs. 3B–D. Schild regression analysis generated a straight line with a slope of 0.92, which was not significantly different from unity (95% CIs: 0.52–1.31, \( n \) = 13) (Fig. 3E). This indicates that noradrenaline-induced relaxation was antagonized competitively by bupranolol (3 × 10^{-7}–3 × 10^{-6} M)
in the presence of $10^{-6}$ M propranolol. The $pA_2$ value of bupranolol was calculated to be 6.64 (95% CIs: 6.40–7.17, $n = 13$).

Figure 4 shows the effects of SR 59230A on noradrenaline-induced relaxation in the presence of propranolol ($10^{-6}$ M). The noradrenaline-induced relaxation was inhibited by SR 59230A ($10^{-7}$–$10^{-6}$ M) in a concentration-dependent manner, shifting the corresponding concentration-response curve to the right (Figs. 4A–C). Figure 4D shows the Schild plot of SR 59230A against noradrenaline based on the results of Figs. 4A–C. Schild regression analysis produced a straight line with a slope of 0.95, which was not significantly different from unity (95% CIs: 0.40–1.50, $n = 12$) (Fig. 4D). This indicates that noradrenaline-induced relaxation was antagonized competitively by SR 59230A ($10^{-7}$–$10^{-6}$ M) in the presence of $10^{-6}$ M propranolol. The $pA_2$ value of SR 59230A was calculated to be 7.27 (95% CIs: 6.92–8.38, $n = 12$).

**Effects of Noradrenaline Metabolites on Methacholine (3×$10^{-5}$ M)-Induced Contraction** Noradrenaline is metabolized by MAO-A and COMT. In this series of experiments, we investigated the effects of six metabolites of noradrenaline (i.e., NMN, DOMA, DHPG, VMA, MHPG, and MHPG-S) in order to determine whether these metabolites can induce a UBSM relaxation response via $\beta_3$-adrenoceptor stimulation. In the presence of clorgiline (a MAO-A inhibitor, $10^{-5}$ M) and 3,5-dinitrocatechol (a COMT inhibitor, $2\times10^{-6}$ M), noradrenaline ($10^{-4}$ M) elicited a relaxation response as shown in Fig. 5A (white column). Noradrenaline ($10^{-4}$ M)-induced relaxation was not further augmented by isoprenaline ($10^{-4}$ M) (Fig. 5A, black column).

In contrast, none of the 6 metabolites (NMN, DOMA, DHPG, VMA, MHPG, or MHPG-S, $10^{-4}$ M each) induced a relaxation response (Figs. 5B–G). However, NMN ($10^{-4}$ M) augmented the methacholine-induced ($3\times10^{-5}$ M) contraction of the UBSM preparation by approximately 10% (Fig. 5B).

**DISCUSSION**

In this study, we investigated whether noradrenaline could be a ligand for the $\beta_3$-adrenoceptor by pharmacological identification of the $\beta$-adrenoceptor subtypes that trigger relaxation responses to noradrenaline in rat UBSM. We also examined 6 metabolites of noradrenaline (i.e., NMN, DOMA, DHPG, VMA, MHPG, and MHPG-S) in order to determine whether they are able to induce UBSM relaxation responses via $\beta_3$-adrenoceptor stimulation. Our pharmacological studies indicated that the predominant $\beta$-adrenoceptor subtype to mediate noradrenaline-induced relaxation is $\beta_3$, and thus, noradrenaline was suggested to be a ligand for the $\beta_3$-adrenoceptor in rat UBSM. In contrast, since none of the noradrenaline metabolites showed a relaxation response, these metabolites are not ligands for the $\beta_3$-adrenoceptor.

First, we will discuss the possible $\beta$-adrenoceptor subtypes in rat UBSM. In the RT-PCR experiment, we detected mRNA expression of all 3 $\beta$-adrenoceptor subtypes ($\beta_1$, $\beta_2$, and $\beta_3$) (Fig. 1). This result supports the findings in previous reports. In those reports, in rat UBSM, all three $\beta$-adrenoceptor subtypes were suggested to have functional significance, as supported by the following pharmacological findings: 1) rat UBSM was relaxed substantially by selective agonists for each subtype (i.e., T-0509 for $\beta_1$, terbutaline for $\beta_2$, and BRL 37344A for $\beta_3$), and 2) a subtype non-selective agonist for $\beta$-adrenoceptors (isoprenaline) was significantly
inhibited by selective antagonists for each subtype (i.e., metoprolol for β₁, butoxamine or ICI-118,551 for β₂, and SR 59230A for β₃).16–18) Our biochemical results and the previous mechanical studies with chemically-synthesized β-adrenoceptor agonists suggest that all 3 β-adrenoceptor subtypes (β₁, β₂, and β₃) could be the potential target for noradrenaline, and thus further pharmacological studies using subtype-selective antagonists are warranted.

Next, we will discuss the participation of β₁- and β₂-adrenoceptors in noradrenaline-induced relaxation. Noradrenaline-induced relaxation was not significantly inhibited by the following β-adrenoceptor antagonists (Fig. 2): atenolol (10⁻⁶ M), a selective β₁-adrenoceptor antagonist at this concentration; ICI-118,551 (3 × 10⁻⁸ M), a selective β₂-adrenoceptor antagonist at this concentration; propranolol (10⁻⁷ M), a β₁- and β₂-adrenoceptor antagonist at this concentration; and bupranolol (10⁻⁷ M), a β₁- and β₂-adrenoceptor antagonist at this concentration. The pA₂ values of each antagonist were previously calculated to be 7.01 (atenolol for β₁),19) 8.47 (propranolol for β₁),20) 8.94 (bupranolol for β₁),21) 8.83 (ICI-118,551 for β₂),22) 8.43 (propranolol for β₂), and 8.60 (bupranolol for β₂).23) Therefore, if either β₂-adrenoceptor subtype significantly contributes to noradrenaline-induced relaxation, the relaxation response should have been inhibited to some extent by these antagonists. However, noradrenaline-induced relaxation was not affected by any of the tested antagonists, thus β₁ and β₂ to this relaxation have been excluded.

Next, we will discuss the possible participation of β₃-adrenoceptors in noradrenaline-induced relaxation. First, noradrenaline-induced relaxation was shown to be competitively antagonized by bupranolol (3 × 10⁻⁶–3 × 10⁻⁷ M), with a pA₂ value of 6.64 (95% CI: 6.40–7.17) (Figs. 3B–E). This pA₂ value (6.64) was deemed to be nearly identical to the values in previous reports: 6.56 against BRL37344-induced relaxation in isolated ileal longitudinal smooth muscle from guinea pigs,12) and 6.70 against CGP 12,177-induced lipolysis in white fat cells from rats.24) Second, noradrenaline-induced relaxation was shown to be competitively antagonized by SR 59230A (10⁻⁷–10⁻⁶ M), with a pA₂ value of 7.27 (95% CI: 6.92–8.38) (Fig. 4). This pA₂ value (7.27) was similar to values that were previously reported: 7.58 against BRL37344-induced relaxation in isolated jejunal longitudinal smooth muscle from rabbits,13) and 6.89 against CL 316,243-induced lipolysis in white fat cells from rats.24) These findings suggest a significant contribution of β₃-adrenoceptors, which are sensitive to both bupranolol and SR 59230A, to noradrenaline-induced relaxation in rat UBSM, and thus noradrenaline could be a ligand for β₃-adrenoceptors in this smooth muscle.

In our study, the pharmacological detection of β₃-adrenoceptors with bupranolol and SR 59230A in noradrenaline-induced relaxation was carried out in the presence of 10⁻⁶ M propranolol, which is similar to the conditions employed in previous reports.12,13) In the absence of propranolol, the slope of the Schild plot regression line for bupranolol
(3 × 10⁻⁷–3 × 10⁻⁶ M) against noradrenaline was far less than unity; thus, we could not calculate the pA₂ value for bupranolol, necessitating the inclusion of 10⁻⁶ M propranolol (data not shown).

In rat UBSM, all three β-adrenoceptors have been shown to be functional, and bupranolol was reported to competitively inhibit isoprenaline-induced relaxation with a pA₂ value of 8.98. This corresponds to the value for β₁ or β₂.⁹⁻¹³

---

**Fig. 4. Effect of SR 59230A on Noradrenaline-Induced Relaxation in Rat Urinary Bladder Smooth Muscle (UBSM)**

A–C: Effects of SR 59230A (10⁻⁷–10⁻⁶ M) on the concentration-response curves for noradrenaline-induced relaxation in the presence of propranolol (10⁻⁶ M). D: Schild plot of the SR 59230A versus noradrenaline analyses shown in A–C. Data are presented as means ± S.E.M., n = 4. Slope and pA₂ values (part D) are presented as means with 95% confidence intervals (95% CIs).

**Fig. 5. Effect of Noradrenaline (A) and 6 Noradrenaline Metabolites (B–G) on Methacholine (3 × 10⁻⁵ M)-Induced Contraction in Rat Urinary Bladder Smooth Muscle (UBSM) in the Presence of Clorgiline (Monoamine Oxidase A (MAO A) Inhibitor, 10⁻⁵ M) and 3,5-Dinitrocatechol (Catechol-O-methyltransferase (COMT) Inhibitor, 2 × 10⁻⁶ M)**

Tested noradrenaline metabolites (10⁻⁴ M) are normetanephrine (NMN; B), 3,4-dihydroxyamandelic acid (DOMA; C), 3,4-dihydroxyphenylglycol (DHPG; D), 4-hydroxy-3-methoxymandelic acid (VMA; E), 4-hydroxy-3-methoxyphenyl glycol (MHPG; F), and 4-hydroxy-3-methoxyphenylglycol sulfate (MHPG-S; G). Data are presented as means ± S.E.M., n = 4. ISO: isoprenaline.
but not to that for β3. Therefore, since bupranolol binds non-selectively to all 3 subtypes (β1, β2, and β3) in rat UBSM and noradrenaline binds selectively to the β1-adrenoceptor, competitive antagonism of bupranolol against noradrenaline would not be expected to occur in the absence of propranolol to block β1 and β2. In contrast, in the presence of propranolol (10^{-6} M), both β1 and β2 are occupied by propranolol, which enables bupranolol to selectively bind to β3 and competitively antagonize noradrenaline. Thus, the slope of the Schild plot regression line for bupranolol versus noradrenaline becomes unity, which enables the pA2 value to be calculated.

However, propranolol at 10^{-6} M shifted the concentration-response curve for noradrenaline-induced relaxation to the right by ca. 2-fold (Fig. 2E). This finding is consistent with previous reports that the pA2 value of propranolol for the β1-adrenoceptor is approximately 6.26,27 Therefore, it is possible that this concentration (10^-6 M) of propranolol is able to inhibit β3-adrenoceptors to some extent in addition to inhibiting β1 and β2, although a control experiment was also performed in the presence of propranolol (10^-6 M) and the pA2 values for bupranolol and SR 59230A were almost identical to the previously reported values.26,27

Finally, we will discuss the results of the noradrenaline metabolites. There were several reasons why we chose to examine their effects. Noradrenaline had been suggested as a metabolite of noradrenaline, was shown to have positive action in guinea pig atrial muscle, and MHPG, another metabolite of noradrenaline, was shown to inhibit lymphocyte chemotaxis similarly to the action of noradrenaline. However, none of the six noradrenaline metabolites (i.e., NMN, DOMA, DHPG, VMA, MHPG, and MHPG-S) showed relaxation responses in our study (Fig. 5). Therefore, these metabolites are unlikely endogenous agonists of the β3-adrenoceptor in rat UBSM; the endogenous agonist is most likely noradrenaline itself. Although NMN stimulated contraction instead of relaxation, we currently do not understand this phenomenon, which should be examined in future studies.

CONCLUSION

Noradrenaline, but not its metabolites, may be a ligand for β3-adrenoceptors to produce relaxation responses in the UBSM of rats. However, our present study was performed with exogenously applied, and not endogenous, noradrenaline. Therefore, further studies are required to examine whether β3-adrenoceptors are targets for endogenous noradrenaline.

Acknowledgments This work was partly supported by The Research Grants of Toho University Faculty of Pharmaceutical Sciences.

Conflict of Interest The authors declare no conflict of interest.

REFERENCES

21) Tanaka Y, Yamashita Y, Michikawa H, Horinouchi T, Koike K.


