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Inhibitory Effect of Fentanyl on Phenylephrine-Induced Contraction of the Rat Aorta

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Purpose: Fentanyl was reported to inhibit the α_1-adrenoceptor agonist-induced contraction. The goal of this *in vitro* study was to identify the α_1-adrenoceptor subtype primarily involved in the fentanyl-induced attenuation of phenylephrine-induced contraction in isolated endothelium-denuded rat aorta. **Materials and Methods:** Aortic rings were suspended in order to record isometric tension. Concentration-response curves for phenylephrine (10^{-9} to 10^{-5} M) were generated in the presence or absence of one of the following drugs: fentanyl (3×10^{-7}, 10^{-6}, 3×10^{-6} M), 5-methylurapidil (3×10^{-8}, 10^{-7}, 3×10^{-7} M), chloroethylclonidine (10^{-5} M) and BMY 7378 (3×10^{-9}, 10^{-8}, 3×10^{-8} M). Phenylephrine concentration-response curves were generated in the presence or absence of fentanyl in rings pretreated with either 3×10^{-9} M prazosin, 10^{-9} M 5-methylurapidil or 3×10^{-9} M BMY 7378. **Results:** Fentanyl (10^{-6}, 3×10^{-6} M) attenuated phenylephrine-induced contraction in the rat aorta. 5-Methylurapidil and BMY 7378 produced a parallel rightward shift in the phenylephrine concentration-response curve. The pA_2 values for 5-methylurapidil and BMY 7378 were estimated to be 7.71 ± 0.15 and 8.99 ± 0.24, respectively. Fentanyl (10^{-6} M) attenuated phenylephrine-induced contraction in rings pretreated with 10^{-9} M 5-methylurapidil, but did not alter the rings when pretreated with 3×10^{-9} M BMY 7378. Pretreatment of the rings with chloroethylclonidine showed a 72.9 ± 2.3% reduction in phenylephrine-induced maximal contraction. **Conclusion:** The results suggest that fentanyl attenuates phenylephrine-induced contraction by inhibiting the pathway involved in the α_{1D}-adrenoceptor-mediated contraction of the rat aorta.

Key Words : Fentanyl, phenylephrine, 5-methylurapidil, BMY 7378, prazosin, rat aorta

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INTRODUCTION

High-dose fentanyl has been widely used as a general anesthetic for patients undergoing heart surgery. Fentanyl administered intravenously at the clinical dose (4.5 μg/kg) has been shown to slightly decrease systemic blood pressure and vascular resistance, whereas high doses (40-160 μg/kg) have been shown to significantly decrease mean peripheral blood pressure.[1,2] In addition, the hypotension produced by high-dose fentanyl (75 μg/kg) is associated with an increased need for the use of alpha-adrenergic agonists to maintain blood pressure.[3] Fentanyl has been shown to inhibit the α_1-adrenoceptor agonist-induced contraction in isolated aorta and pulmonary arteries.[4-6]

The α_1-adrenoceptors are a heterogeneous group of receptors and, based on radioligand binding, molecular biology and isolated tissue experiments, they have been classified into three subtypes: the α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors for native receptors.[7] All three subtypes of the α_1-adrenoceptors have a high affinity for the non-subtype-selective α_1-adrenoceptor antagonist prazosin,[7] and are expressed in

vascular smooth muscles, including the rat aorta.[8] These subtypes can be identified by selective and non-selective antagonists. For example, 5-methyl-urapidil (5-MU) has a 10 to 50 times higher affinity for α_{1A}-adrenoceptors, and the affinity of BMY 7378 for α_{1D}-adrenoceptors is at least 100-fold higher.[8] Chloroethylclo-nidine (CEC) is an irreversible antagonist that preferentially inactivates α_{1B}-adrenoceptors, but it can also partially inactivate α_{1D}-adrenoceptors.[8] The α_{1D}-adrenoceptors mediate the phenylephrine-induced contraction of the rat abdominal aorta, thoracic aorta, and mesenteric artery,[9,10] and α_{1B}-adrenoceptors mediate the phenylephrine-induced contraction of the human umbilical vein and canine pulmonary artery, as well as phenylephrine-induced contraction in the dog aorta.[6,11,12] However, to the best of our knowledge, the α_1-adrenoceptor subtype that is involved in the fentanyl-induced attenuation of the phenylephrine contraction-response curve in systemic circulation such as rat aortas has not yet been identified. The goals of the current *in vitro* study were to identify the α_1-adrenoceptor subtype that is involved mainly in the fentanyl-induced attenuation of phenylephrine-induced contraction in isolated endothelium-denuded rat aorta and to characterize the α_1-adrenoceptor subtype that is functionally important in mediating the contractile response to phenylephrine.

MATERIALS AND METHODS

All experimental procedures and protocols were approved by the Institutional Animal Care and Use Committee. Sprague-Dawley male rats, weighing 250-350 g each, were anesthetized by the intraperitoneal administration of pentobarbital sodium (50 mg/kg). The descending thoracic aorta was dissected free, and the surrounding connective tissue and fat were removed under microscopic guidance while the blood vessels were bathed in Krebs solution of the following composition: 118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 2.4 mM $CaCl_2$, 25 mM $NaHCO_3$, 11 mM glucose and 0.03 mM EDTA. The aorta was then cut into 2.5-mm rings, which were suspended on Grass isometric transducers (FT-03, Grass Instrument, Quincy, MA, USA) with a 2.0 g resting tension in 10 mL temperature-controlled baths (37°C) containing the Krebs solution, which was continuously gassed with 95% O_2 and 5% CO_2. The rings were equilibrated at a 2.0 g resting tension for 120 min, during which time the bathing solution was changed every 15 min. In all rings, the endothelium was intentionally removed by inserting a 25-G needle tip into the lumen of the rings and gently rolling the rings for a few seconds. The contractile response induced by isotonic 60 mM KCl was measured in each of the aortic rings.

Experimental protocol

The first series of these experiments was aimed at assessing the effect of fentanyl on contractile response induced by the α_1-adrenoceptor agonist phenylephrine in endothelium-denuded rings. Fentanyl was added directly to the organ bath 30 min before cumulative phenylephrine-induced contraction. The effect of fentanyl on the concentration-response curves for phenylephrine (10^{-9} to 10^{-5} M) was assessed by comparing the contractile response in the presence or absence of fentanyl (3×10^{-7}, 10^{-6}, 3×10^{-6} M).

The second series of experiments was designed to determine which subtype of α_1-adrenoceptor is functionally important in mediating phenylephrine-induced contraction in endothelium-denuded rat aorta. The effect of subtype-selective α_1-adrenoceptor antagonists (α_{1A}-adrenoceptor antagonist: 3×10^{-8}, 10^{-7}, 3×10^{-7} M 5-MU; α_{1D}-adrenoceptor antagonist: 3×10^{-9}, 10^{-8}, 3×10^{-8} M BMY 7378) on the concentration-response curve for phenylephrine was assessed by comparing each contractile response in the presence and absence of each subtype-selective α_1-adrenoceptor antagonist. The incubation period for each subtype-selective α_1-adrenoceptor antagonist was 30 min before phenylephrine-induced contraction.

The third series of experiments was designed to assess the effect of the irreversible α_{1B}-adrenoceptor antagonist CEC on the concentration-response curve for phenylephrine. The first concentration-response curve for phenylephrine was constructed before pretreatment with 10^{-5} M CEC. After being washed, aortic rings were exposed to CEC (10^{-5} M) for a period of 20 min. Following removal of the CEC by exchanges of the Krebs solution every 10 min for 1 hour, a second concentration-response curve for phenylephrine was constructed.

In the fourth series of experiments, the α_1-adrenoceptor subtype dependence of fentanyl-induced attenuation of the contractile response induced by phenylephrine was examined. The effect of fentanyl (10^{-6} M) on the concentration-response curve for phenylephrine in the rings which had been pretreated with either 10^{-9} M 5-MU or 3×10^{-9} M BMY 7378 was assessed by comparing the contractile response in the presence and absence of fentanyl (10^{-6} M). In addition, the role of the α_1-adrenoceptor in the fentanyl-induced attenuation of the contractile response induced by phenylephrine was assessed by examining the phenylephrine (10^{-9} to 10^{-4} M) concentration-response curve after prazosin (3×10^{-9} M) was added directly to organ bath, either alone or in combination with fentanyl (3×10^{-6} M). The incubation period for the subtype-selective or non-subtype-selective α_1-adrenoceptor antagonists (3×10^{-9} M prazosin, 10^{-9} M 5-MU and 3×10^{-9} M BMY 7378) plus fentanyl (10^{-6}, 3×10^{-6} M) or α_1-adrenoceptor antagonist (subtype-selective or non-subtype-selective) alone was 30 min before the phenylephrine-induced contraction.

Drug and solution

All drugs used in the present study were of the highest purity commercially available and included phenylephrine HCl, acetylcholine, prazosin, 5-MU, CEC, BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro [4.5]decane-7,9-dione dihydrochloride) (Sigma Chemical, St. Louis, MO, USA), and fentanyl (Hana Pharmaceutical Co., Ltd., Seoul, Korea). All concentrations are expressed as the final molar concentration in the organ bath. All drugs were dissolved in distilled water.

Data analysis

The values are expressed as means ± SD. Contractile responses to phenylephrine are expressed as a percentage of their own maximum contraction to isotonic 60 mM KCl. The first and second phenylephrine-induced contractions are expressed as a percentage of the maximum obtained from the first phenylephrine concentration-response curve. The logarithm of the drug concentration (ED_{50}), eliciting 50% of the maximum contractile response, was calculated using nonlinear regression analysis by fitting the concentration-response relation for phenylephrine to a sigmoidal curve, by using commercially available software (Prism version 3.02: GraphPad software, San Diego, CA, USA). Data were fitted to a sigmoidal dose-response curve using the following algorithm Y = Bottom + (Top - Bottom)/(1 + 10^ ((LogED$_{50}$ - X) × Hill Slope)). The concentration ratio (CR) is defined as the concentration of agonist required to induce 50% maximal contractile response in the presence of antagonist divided by the agonist concentration that elicits the same degree of response in the absence of antagonist. The pA_2 value represents the concentration of antagonists necessary to displace the concentration-response curve of an agonist by twofold. Subtype-selective α_1-adrenoceptor antagonist pA_2 values (-log M) were calculated from Arunlakshana and Schild plots and were obtained from the X-intercept of the plot of log (CR-1) against log molar antagonist concentration, where the slope was not different from unity.[13] The slope and pA_2 values calculated from Arunlakshana and Schild plots are expressed as mean ± SEM.[13] Statistical analysis was performed using Student's t-test for paired comparison. One-way analysis of variance, followed by Tukey's multiple comparison, was used to compare more than two means. The p value < 0.05 was considered

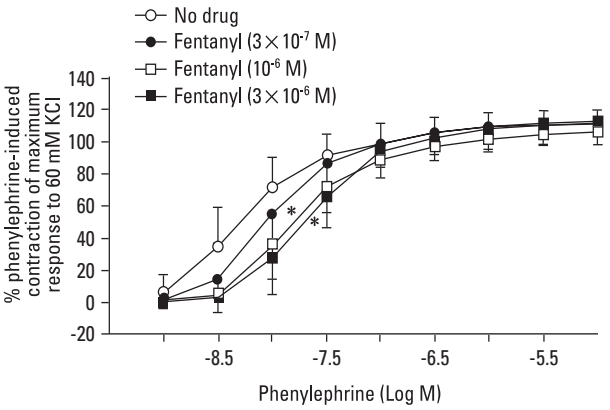

Fig. 1. Effect of fentanyl on the phenylephrine dose-response curve in endothelium-denuded rat aorta. Fentanyl (10^{-6}, 3×10^{-6} M) produced a significant rightward shift (ED_{50}: *p < 0.05 versus no drug) in the phenylephrine dose-response curve. Data are shown as mean ± SD and expressed as the percentage of maximal contraction induced by isotonic 60 mM KCl (isotonic 60 mM KCl-induced contraction: 100% = 2.62 ± 0.39 g [n = 7], 100% = 2.65 ± 0.33 g [n = 5], 100% = 2.67 ± 0.37 g [n = 7] and 100% = 2.22 ± 0.29 g [n = 6] for the rings not treated with fentanyl, the fentanyl [3×10^{-7} M], [10^{-6} M] and [3×10^{-6} M] pretreated rings, respectively).

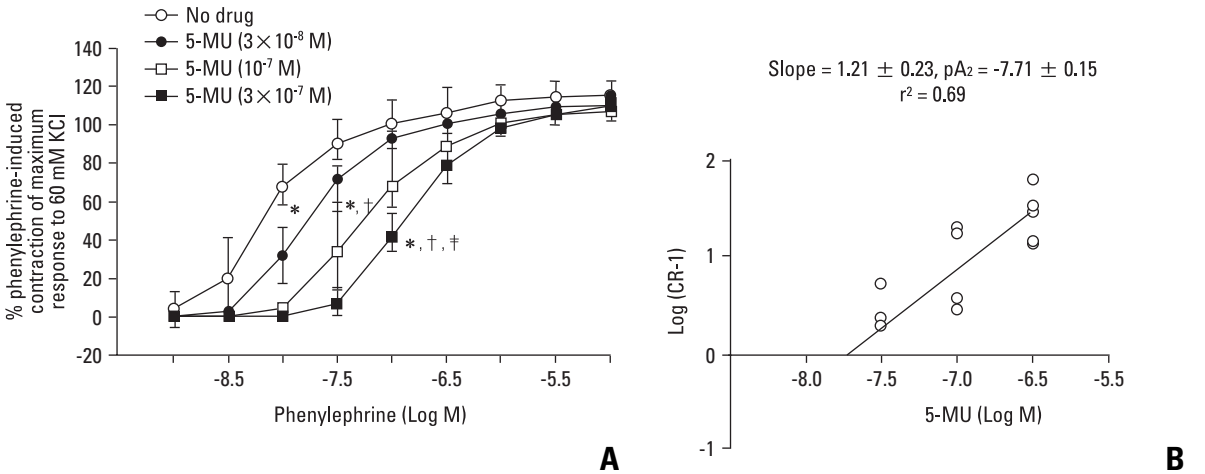

Fig. 2. (A) Effect of 5-methylurapidil (5-MU) on phenylephrine doe-response curve. 5-MU (3×10^{-8}, 10^{-7}, 3×10^{-7} M) produced a parallel rightward shift (ED_{50}: *p < 0.01 versus no drug, †p < 0.001 versus 3×10^{-8} M 5-MU, ‡p < 0.05 versus 10^{-7} M 5-MU) in the phenylephrine dose-response curve in a concentration-dependent manner. Data are shown as mean ± SD and expressed as the percentage of maximal contraction induced by isotonic 60 mM KCl (isotonic 60 mM KCl-induced contraction: 100% = 3.30 ± 0.84 g [n = 5], 100% = 2.82 ± 0.75 g [n = 5], 100% = 2.74 ± 0.52 g [n = 5] and 100% = 2.38 ± 0.71 g [n = 5] for the rings not treated with 5-MU, the 5-MU [3×10^{-8} M], [10^{-7} M] and [3×10^{-7} M] pretreated rings, respectively). (B) A Schild plot was constructed with the concentration ratio (CR: ED_{50} in the presence and absence of 5-MU) for individual experiments. The slope of the Schild plot for 5-MU was 1.21 ± 0.23 (r^2 = 0.69), and the concentration (-log M) of 5-MU necessary to displace the phenylephrine concentration-response curve by twofold was 7.71 ± 0.15.

significant. N refers to the number of rats whose descending thoracic aortic rings were used in each experiment. Each group contained at least two rings from the same rat.

RESULTS

Fentanyl (3×10^{-7} M) did not significantly alter the phenylephrine concentration-response curve (Fig. 1). However, higher concentration of fentanyl (10^{-6}, 3×10^{-6} M) significantly attenuated ($p < 0.05$ versus no drug) phenylephrine-induced contraction (ED_{50}: no drug; -8.41 ± 0.34 versus 10^{-6} M fentanyl; -7.84 ± 0.29, 3×10^{-6} M fentanyl; -7.70 ± 0.28) in endothelium-denuded rat aorta (Fig. 1).

Treatment of the aorta with 5-MU (3×10^{-8}, 10^{-7}, 3×10^{-7} M) caused a parallel rightward shift ($p < 0.01$ versus no drug) in the phenylephrine concentration-response curve (ED_{50}: no drug; -8.25 ± 0.19 versus 3×10^{-8} M 5-MU; -7.81 ± 0.13, 10^{-7} M 5-MU; -7.23 ± 0.26, 3×10^{-7} M 5-MU; -6.82 ± 0.15) in a concentration-dependent manner (Fig. 2A). Analysis of the data using an Arunlakshana and Schild plot for the antagonism of phenylephrine-induced contraction by 5-MU yielded a slope (1.21 ± 0.23) that was not significantly different from unity (Fig. 2B). The pA_2 value for 5-MU was 7.71 ± 0.15 (Fig. 2B), which is approximately 10 times less than the reported affinity (9.3-8.4) for the α_{1A}-adrenoceptor, suggesting that the α_{1A}-adrenoceptor subtype does not play a main role in the phenylephrine-induced contraction of the rat aorta.[8]

BMY 7378 (3×10^{-9}, 10^{-8}, 3×10^{-8} M) produced a parallel rightward shift ($p < 0.01$ versus no drug) in the phenylephrine concentration-response curve (ED_{50}: no drug; -8.26 ± 0.30 versus 3×10^{-9} M BMY 7378; -7.63 ± 0.19, 10^{-8} M BMY 7378; -7.32 ± 0.23, 3×10^{-8} M BMY 7378; -6.92 × 0.14) in a concentration-dependent manner (Fig. 3A). The Arunlakshana and Schild plot for antagonism of phenylephrine-induced contraction by BMY 7378 yielded a slope (0.87 ± 0.19) that was not significantly different from unity (Fig. 3B). The pA_2 value for BMY 7378 was 8.99 ± 0.24 (Fig. 3B), which is close to the reported affinity (8.7-8.1) for the α_{1D}-adrenoceptor, suggesting that the α_{1D}-adrenoceptor plays a primary role in phenylephrine-induced contraction of the rat aorta.[8]

CEC (10^{-5} M) produced 72.9% inhibition of the first phenylephrine-induced maximal contraction ($p < 0.0001$ versus no drug) (Fig. 4), which suggests that phenylephrine-induced contraction in the rat aorta involves the CEC-sensitive α_1-adrenoceptor subtype (α_{1B}- and α_{1D}-adrenoceptors).

Comparison with aortic rings not treated with fentanyl showed that fentanyl (10^{-6} M) significantly attenuated ($p <$ 0.01 versus 5-MU alone) phenylephrine-induced contraction (ED_{50}: 10^{-9} M 5-MU; -8.10 ± 0.24 versus 10^{-9} M 5-MU + 10^{-6} M fentanyl; -7.72 ± 0.24) in rings pretreated with 5-MU at a concentration of 10^{-9} M which is close to the reported affinity (9.3-8.4) for the α_{1A}-adrenoceptor (Fig. 5).[8]

In rings pretreated with BMY 7378 at 3×10^{-9} M concentration which is close to the reported affinity (8.7-8.1) for the α_{1D}-adrenoceptor, fentanyl (10^{-6} M) did not significantly alter phenylephrine-induced contraction as compared with rings not treated with fentanyl (Fig. 6).[8]

Fentanyl (3×10^{-6} M) had no effect on phenylephrine-induced contraction of rings pretreated with prazosin (3×10^{-9} M) (Fig. 7).

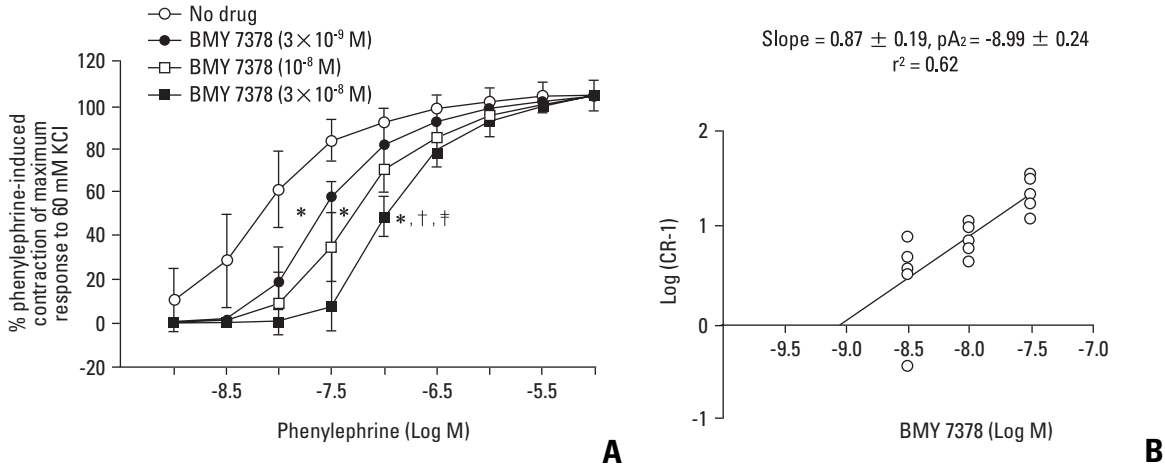

Fig. 3. (A) Effect of BMY 7378 on phenylephrine does-response curve. BMY 7378 (3×10^{-9}, 10^{-8}, 3×10^{-8} M) produced a parallel rightward shift (ED_{50}: *$p < 0.01$ versus no drug, †$p < 0.001$ versus 3×10^{-9} M BMY 7378, ‡$p < 0.001$ versus 10^{-8} M BMY 7378) in the phenylephrine dose-response curve in a concentration-dependent manner. Data are shown as mean ± SD and expressed as the percentage of maximal contraction induced by isotonic 60 mM KCl (isotonic 60 mM KCl-induced contraction: 100% = 3.03 ± 0.81 g [n = 5], 100% = 2.90 ± 0.31 g [n = 5], 100% = 2.47 ± 0.66 g [n = 5] and 100% = 2.34 ± 0.26 g [n = 5] for the rings not treated with BMY 7378, the BMY 7378 [3×10^{-9} M], [10^{-8} M] and [3×10^{-8} M] pretreated rings, respectively). (B) A Schild plot was constructed with the concentration ratio (CR: ED_{50} in the presence and absence of BMY 7378) for individual experiments. The slope of the Schild plot for BMY 7378 was 0.87 ± 0.19 ($r^2 = 0.62$), and the concentration (-log M) of BMY 7378 necessary to displace the phenylephrine concentration-response curve by twofold was 8.99 ± 0.24.

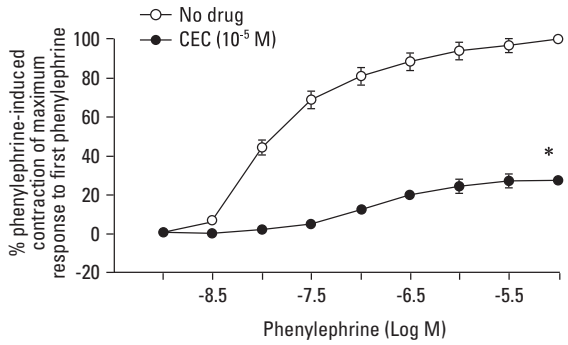

Fig. 4. Effect of chloroethylclonidine (CEC) on phenylephrine dose-response curve in endothelium-denuded rat aorta. The first phenylephrine dose-response curves were obtained before (no drug) exposure to 10^{-5} M CEC. After aortic rings were pretreated with 10^{-5} M CEC for 20 min and washed intensively, second dose-response curves for phenylephrine were obtained. CEC (10^{-5} M) inhibited maximal contraction induced by phenylephrine (*$p < 0.0001$ versus no drug). Data are shown as mean ± SD and expressed as the percentage of the first phenylephrine-induced maximal contraction (phenylephrine-induced maximal contraction: 100% = 3.9 ± 0.16 g [n = 4] for the rings with no drug).

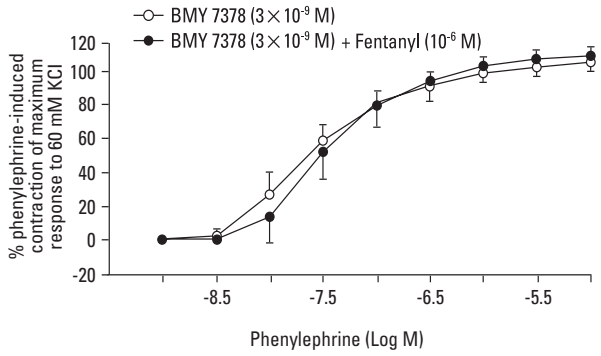

Fig. 6. Effect of 10^{-6} M fentanyl on the phenylephrine dose-response curve in rings pretreated with 3×10^{-9} M BMY 7378. Fentanyl (10^{-6} M) did not significantly alter pheny lephrine-induced contraction compared with rings pretreated with 3×10^{-9} M BMY 7378 alone. Data are shown as mean ± SD and expressed as the percentage of maximal contraction induced by isotonic 60 mM KCl (isotonic 60 mM KCl-induced contraction: 100% = 2.89 ± 0.33 g [n = 7] and 100% = 2.72 ± 0.36 g [n = 7] for the rings not treated with fentanyl and the fentanyl [10^{-6} M] pretreated rings, respectively).

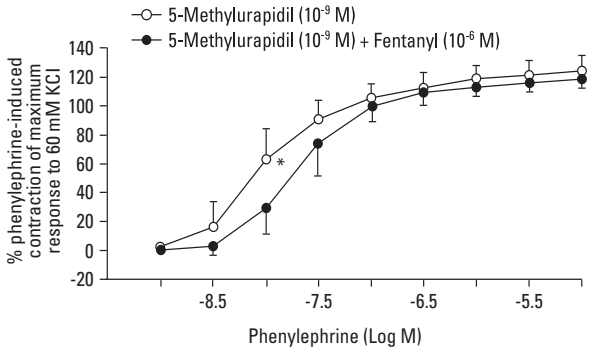

Fig. 5. Effect of 10^{-6} M fentanyl on phenylephrine dose-response curve in rings pretreated with 10^{-9} M 5-methylurapidil. Fentanyl (10^{-6} M) attenuated (ED_{50}: *$p < 0.01$ versus 10^{-9} M 5-methylurapidil alone) phenylephrine-induced contraction compared with rings pretreated with 10^{-9} M 5-methylurapidil alone. Data are shown as mean ± SD and expressed as the percentage of maximal contraction induced by isotonic 60 mM KCl (isotonic 60 mM KCl-induced contraction: 100% = 2.71 ± 0.68 g [n = 9] and 100% = 2.98 ± 0.43 g [n = 9] for the rings not treated with fentanyl and the fentanyl [10^{-6} M] pretreated rings, respectively).

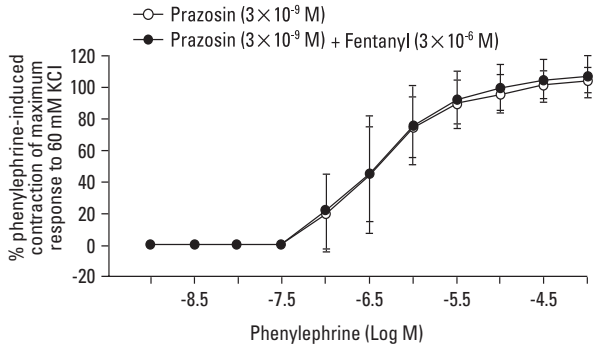

Fig. 7. Effect of fentanyl on the phenylephrine dose-response curve in rings pretreated with 3×10^{-9} M prazosin. Fentanyl (3×10^{-6} M) had no effect on the phenylephrine dose-response curve in the rings pretreated with 3×10^{-9} M prazosin. Data are shown as mean ± SD and expressed as the percentage of maximal contraction induced by isotonic 60 mM KCl (isotonic 60 mM KCl-induced contraction: 100% = 3.06 ± 0.32 g [n = 7] and 100% = 3.03 ± 0.55 g [n = 7] for the rings not treated with fentanyl and the fentanyl [3×10^{-6} M] pretreated rings, respectively).

DISCUSSION

Despite widespread use of fentanyl in patients undergoing heart surgery, we believe that this is the first study to show that fentanyl attenuates phenylephrine-induced contraction by inhibiting the cellular signal transduction pathway involved in the α_{1D}-adrenoceptor-mediated contraction of the rat aortic smooth muscle. The α_{1D}-adrenoceptor exerts a great influence on modulation of contraction induced by phenylephrine in rat aortic smooth muscle.

Fentanyl attenuates phenylephrine-induced contraction in the canine pulmonary artery by binding to the α_{1B}-adrenoceptor,[6] and attenuates also the α_1-adrenoceptor agonist (phenylephrine and norepinephrine)-induced dose-response curve by an alpha-adrenergic blocking action in isolated rabbit and rat aorta.[4,5] Consistent with previous studies,[4-6] fentanyl (10^{-6}, 3×10^{-6} M) attenuated phenylephrine-induced contraction in the present study. In addition, prazosin completely abolished fentanyl-induced attenuation of contractile response induced by phenylephrine. These results suggest that fentanyl attenuates the phenylephrine dose-response curve by inhibiting α_1-adrenoceptor-mediated contraction of rat aortic smooth muscle. The CR which was calculated as the ratio of the ED_{50} for phenylephrine in the presence or absence of fentanyl (10^{-6} M) was 2.52 ± 0.52, suggesting that the dose of phenylephrine required for the same magnitude of phenylephrine-induced contraction in the presence of fentanyl (10^{-6} M) is about 2.5 times higher than that in the absence of fentanyl. Fentanyl (100 ng/mL)

induces a small, but statistically significant attenuation of the phenylephrine concentration-response curve in isolated endothelium-intact canine coronary arteries.[14] However, the present study showed that fentanyl at a concentration of 3×10^{-7} M, which corresponds approximately to the plasma concentration (100 ng/mL) occurring in patients anesthetized with fentanyl for major surgery, had no effect on phenylephrine-induced contraction.[3] The discrepancy in the results of the two studies may be ascribed to the difference in the vessel selected (endothelium-intact canine coronary artery versus endothelium-denuded rat aorta).

The estimated affinity of 5-MU for the α_{1A}-adrenoceptor is approximately 10 times greater than that for the α_{1D}-adrenoceptor and 100 times greater than that for the α_{1B}-adrenoceptor.[15,16] In the present *in vitro* study, the affinity of 5-MU estimated in the rat aorta ($pA_2 = 7.71 \pm 0.15$) was close to the value expected for an interaction with the α_{1D}-adrenoceptor (7.80 ± 0.09, 7.91 ± 0.07) rather than the α_{1A}-adrenoceptor (8.50 ± 0.09, 8.68 ± 0.09) or α_{1B}-adrenoceptor (6.80 ± 0.13, 6.76 ± 0.17).[15,16] BMY 7378 is a selective α_{1D}-adrenoceptor antagonist, whose selectivity for α_{1D}-adrenoceptor is 100 times greater than that for the α_{1A}- or α_{1B}-adrenoceptor.[15,17] In this study, the estimated affinity of BMY 7378 was 8.99 ± 0.24, which is close to pKi values for the human recombinant α_{1D}-adrenoceptor expressed in rat-1 fibroblast (9.39 ± 0.05) and the rat aorta (8.88 ± 0.10), demonstrating predominance of the α_{1D}-adrenoceptor subtype.[18] These results suggest that phenylephrine-induced contraction in rat aortic smooth muscle is primarily mediated by the α_{1D}-adrenoceptor. The agreement between agonist potency and affinity for the α_{1D}-adrenoceptor binding site shows that the α_{1D}-adrenoceptor is responsible for mediating contraction of the rat aorta.[19] The phenylephrine-induced contraction of the rat thoracic aorta is mainly mediated by the α_{1D}-adrenoceptor.[9,20,21] Consistent with previous studies,[9,19-21] the present results suggest that the α_{1D}-adrenoceptor is functionally important in mediating the phenylephrine-induced contraction of the rat aorta. Fentanyl (10^{-6} M) attenuated phenylephrine-induced contraction in rings when pretreated with 10^{-9} M 5-MU, but it did not significantly alter phenylephrine-induced contraction in rings pretreated with 3×10^{-9} M BMY 7378. Together with the present results suggesting that the α_{1D}-adrenoceptor is mainly involved in phenylephrine-induced contraction, these results suggest that fentanyl attenuates phenylephrine-induced contraction by inhibiting α_{1D}-adrenoceptor-mediated contraction in the rat aorta. However, to lessen overestimation that may occur from concentration of 5-methylurapidil (10^{-9} M) and BMY 7378 (3×10^{-9} M) adopted from previously reported pA_2 values, competitive binding studies with radioisotope-tagged drugs are needed to examine the direct effect of fentanyl on α_1-adrenoceptor subtypes (α_{1A} and α_{1D}).[8] In a radioligand binding study involving 20-min incubation at 37°C, the α_{1B}-adrenoceptor was preferentially (89 to 98%) inactivated by the irreversible α_{1B}-adrenoceptor antagonist (10^{-4} M CEC), followed by α_{1D}-adrenoceptor at 75 to 86% and α_{1A}-adrenoceptor at 11 to 18%.[22] Rat aortic rings exposed to CEC (5×10^{-6} M) show 20% reduction in phenylephrine-induced contraction.[23] However, inhibition of contraction by pretreatment with 10^{-5} M CEC (20 min) was 72.9 ± 2.3% in this *in vitro* study. This different effect of CEC on phenylephrine-induced contraction may be ascribed to the difference in the concentration of CEC, incubation period and washing method with fresh Krebs solution. CEC has been used extensively as a tool to discriminate α_{1A}-adrenoceptor (CEC-insensitive) from α_{1B}- and α_{1D}-adrenoceptors (CEC-sensitive).[8] Pretreatment of rat aorta with CEC (3×10^{-5} M) showed 83.9% reduction of phenylephrine-induced contraction, which suggests that α_1-adrenoceptor subtype of the rat aorta is CEC-sensitive subtype (α_{1B}- and α_{1D}-adrenoceptors).[24] Based on the findings of current and previous studies, CEC-sensitive α_{1D}-adrenoceptor appears to be involved in the phenylephrine-induced contraction of the rat aorta.[8,24] Interestingly, α_1-adrenoceptors seem to be regulated by crosstalk between G-protein-coupled receptors and subsequent signaling cascades.[25] Further investigation is needed in order to determine the effect of fentanyl on G-protein, phospholipase C, the coupling processes, inositol 1,4,5-triphosphate and diacylglycerol, since all these factors are involved in the cellular signal transduction pathway for α_{1D}-adrenoceptor-mediated phenylephrine-induced contraction.

Fentanyl (2.97×10^{-6} M) attenuates the nitric oxide-mediated relaxation induced by acetylcholine,[26] and produces hypotension by inhibiting central sympathetic outflow in intact dogs anesthetized with enflurane.[27] Previous studies have shown that fentanyl has no effect on myocardial contractility at a clinical dose and increases myocardial contractile force at supraclinical doses.[28,29] According to the findings described above, the net hemodynamic effect of fentanyl *in vivo* is a composite of the effect of fentanyl on blood vessel, central sympathetic activity and the heart.[26-29] Any clinical implications of fentanyl on regional hemodynamics must be tempered by the fact that a large conduit artery, the aorta, was used in this *in vitro* experiment, whereas the resistance vessels with the arterioles of a diameter of 100-300 μm control most organ blood flow.[30] Even with these limitations, since 3×10^{-7} M fentanyl (plasma fentanyl concentration 100 μg/mL) did not alter phenylephrine-induced contraction, the indirect mechanism for vasodilation, which is mediated by decreased central sympathetic outflow induced by fentanyl, might be involved in the hypotension observed in the previous *in vivo* studies.[1-3,27]

Fentanyl significantly attenuated phenylephrine-induced contraction at concentrations of 10^{-6} and 3×10^{-6} M, which

are higher than clinically relevant concentration (9.52×10^{-8} M).[3] However, the rapid redistribution of fentanyl (octanol: water partition coefficient = 813) to lipid-rich tissue may create a discrepancy between the serum concentration and actual tissue concentration.[31] Because cerebrospinal fluid contains very little protein in comparison to plasma, the average concentration of active fentanyl in cerebrospinal fluid is approximately 46% of the total plasma fentanyl concentration, which is more than twice the free fraction of plasma fentanyl.[32,33] Small changes in the amount or binding capacity of proteins in certain pathologic conditions (example: liver disease, hemodilution, hypoproteinemia) could result in an increase in the free fraction of fentanyl. Taking the above findings into consideration, the 10^{-6} M concentration of fentanyl required for an inhibitory effect on phenylephrine-induced contraction might be the concentration encountered in clinical settings.[31-33]

In conclusion, the present results suggest that fentanyl at supraclinical dose (10^{-6} M) attenuates phenylephrine-induced contraction of rat aortic smooth muscle by inhibiting the pathway involved in the α_{1D}-adrenoceptor-mediated contraction. In addition, the α_{1D}-adrenoceptor is functionally important in mediating phenylephrine-induced contraction of isolated rat aorta.

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