

Bidirectional effects of dexmedetomidine on human platelet functions *in vitro*

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ABSTRACT

Platelets express the imidazoline (I)-receptor, I₁ and I₂, as well as the α_2 -adrenoceptor. Although dexmedetomidine, a selective α_2 -adrenoceptor agonist with some affinity for the I-receptor is expected to affect platelet function, the effects of dexmedetomidine on platelet functions remain unclear. In the present study, we investigated the effects of dexmedetomidine on human platelet functions *in vitro*. The effects of dexmedetomidine on platelet aggregation were examined using aggregometers. The formation of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) in platelets was measured by an enzyme immunoassay. In addition, P-selectin expression in platelets was estimated by flow cytometry. Dexmedetomidine enhanced and suppressed platelet aggregation in the absence and the presence of yohimbine, an α_2 -antagonist, respectively. Efaroxan, an I₁-antagonist, and methylene blue, a soluble guanylate cyclase inhibitor, abolished the suppressive effect of dexmedetomidine, whereas idazoxan, an I₂-antagonist, showed no effect. Dexmedetomidine suppressed cAMP formation and enhanced P-selectin expression in platelets, and these effects were inhibited by yohimbine.

Dexmedetomidine increased cGMP formation in platelets in the presence of yohimbine, and this increase was suppressed by efaroxan. These results demonstrated that dexmedetomidine has both enhancing and suppressive effects on human platelet functions through its action on the α_2 -adrenoceptor and on the I₁-receptor, respectively.

Key Words: Dexmedetomidine, Platelet, α_2 -adrenoceptor, Imidazoline receptor

1. Introduction

Because platelets are essential for maintaining hemostasis, information on the effects of perioperatively used drugs including anaesthetics on platelet functions is indispensable for patient care. Previously, the inhibitory effects of volatile anaesthetics on human platelet aggregation were shown in both *in vitro* (Hirakata et al., 1995; Hirakata et al., 1996) and *in vivo* studies (Hirakata et al., 1997). With respect to intravenous anaesthetics, we have reported that propofol has both enhancing and suppressive effects on human platelet aggregation *in vitro* (Hirakata et al., 1999), and ketamine inhibits human platelet aggregation possibly by suppression of inositol trisphosphate formation (Nakagawa et al., 2002). However, the effects of a number of drugs, which have recently become clinically applicable, on platelet functions remain to be examined.

It has not been extensively examined whether dexmedetomidine, an R (+)-enantiomer of medetomidine with a highly selective affinity for the α_2 -adrenoceptor, that is clinically used for sedation and analgesia (Kamibayashi and Maze, 2000; Ramsay and Luterman, 2004), affects human platelet function. As it was reported that adrenaline can potentiate platelet aggregation by activation of the α_2 -adrenoceptor in platelets (Lanza and Cazenave, 1985), it is probable that dexmedetomidine also affects platelet functions. Dexmedetomidine also exhibits some affinity for imidazoline (I)-receptors, which are expressed in various cells, including platelets, and are suggested to participate in many physiological functions (Dahmani et al., 2008; Ernsberger et al., 1997a; Ernsberger and Haxhiu, 1997b; Savola and Savola, 1996; Virtanen et al., 1988; Wikberg

et al., 1991). The existence of I_1 - and I_2 -receptors in human platelets (Michel et al., 1990; Piletz and Sletten, 1993; Piletz et al., 1996), and the suppressive effect of imidazoline agents on rabbit platelet aggregation *via* I -receptors (Yokota et al., 2013), may suggest that dexmedetomidine can affect human platelet functions *via* I -receptors. It is important clinically as well as pharmacologically to examine the effect of dexmedetomidine on platelet functions, because dexmedetomidine is often administered to critically ill patients whose platelet number in the blood is seriously decreased by a variety of pathophysiological mechanisms,.

The aim of this study was to evaluate the effects of dexmedetomidine on human platelet functions and to elucidate the underlying mechanisms especially concerning the α_2 -adrenoreceptor and I -receptors. Our results demonstrate that dexmedetomidine has both enhancing and suppressive effects on human platelet functions through action on the α_2 -adrenoceptor and the I_1 -receptor, respectively.

2. Materials and methods

2.1. Platelet preparation

Written informed consent was obtained from subjects, and the protocol was approved by the ethics committee of Kyoto University Hospital. Venous blood was obtained by venipuncture of antecubital veins from 15 healthy volunteers who had not taken any medication for at least two weeks before blood sampling. The blood was mixed with 10% volume of 3.8% tri-sodium citrate. Platelet-rich plasma (PRP) was prepared by centrifugation of the blood sample at 160 *g* for 10 min at room temperature and collection of the supernatant. The remaining lower portion was further centrifuged at 1600 *g* for 30 min at room temperature, and the clear supernatant was used as platelet-poor plasma (PPP).

2.2. Chemicals and drugs

Dexmedetomidine and idazoxan were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Yohimbine and methylene blue were purchased from Nacalai Tesque (Kyoto, Japan). Levomedetomidine, an S (-)-enantiomer of medetomidine was kindly supplied by Orion Corporation (Espoo, Finland). Adenosine diphosphate (ADP) and efaroxan were purchased from Sigma-Aldrich (St. Louis, MO, USA), and Tocris Bioscience (Bristol, UK), respectively. 9, 11-epithio-11, 12-methanothromboxane A₂ (STA₂) was provided by Ono Pharmaceutical (Osaka, Japan). Peridinin Chlorophyll Protein (PerCP)-labeled anti-CD61 antibody, Phycoerythrin (PE)-labeled anti-CD62P (P-selectin) antibody and PE-labeled IgG for control were obtained from

Becton Dickinson (San Diego, CA, USA). All other chemicals were of analytical grade. We confirmed that all buffers or solvents for diluting materials in our experiments showed no effect on the results.

2.3. Measurement of agonist-induced and spontaneous platelet aggregation

An aliquot of PRP was pipetted into a cylindrical cuvette containing either dexmedetomidine or levomedetomidine and incubated at 37°C for 3 min in the presence or absence of various antagonists (yohimbine, efaroxan, idazoxan and methylene blue). Then, the sample was stirred constantly at 37°C with a magnetic bar at a rate of 1000 rpm. ADP-induced aggregation was measured for 7 min as a change in light transmission using an aggregometer (MCM Hema Tracer 212; MC Medical, Tokyo, Japan). The light transmission of PPP was taken as 100%, and aggregation was expressed as percentage of the light transmission. Spontaneous platelet aggregation in PRP was measured without any agonists for 7 min using an ultra-sensitive platelet aggregation analyzer (PA-200, Kowa, Tokyo, Japan), and expressed as light scattering intensity.

2.4. Cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) assays

Washed platelets were used in cAMP and cGMP assays to exclude the possible effects of other blood cells on the results. PRP in a 10% volume of 100 mM ethylenediaminetetraacetic acid

(EDTA) was centrifuged at 900 *g* for 15 min at 4°C. The pellet was suspended in the wash buffer containing 8 mM Na₂HPO₄, 2 mM NaH₂PO₄, 10 mM EDTA, 135 mM NaCl and 5 mM KCl, and centrifuged again at 900 *g* for 15 min at 4°C. The platelets were finally suspended at a concentration of either 10⁶ platelets μl⁻¹ or 2.5 x 10⁶ platelets μl⁻¹ for the cAMP and cGMP assays, respectively, in the assay buffer containing 10 mM N-2-Hydroxyethylpiperazine-N'-2'-ethanesulfonic acid (HEPES), 0.5 mM Na₂HPO₄, 145 mM NaCl, 5mM KCl, and 6 mM glucose.

For the cAMP assay, 100 μl of the platelet suspension was incubated at 37°C for 3 min in a cylindrical cuvette, and then stirred with a magnetic bar at a rate of 1,000 rpm at 37°C. Platelets were stimulated with 0.1 μM STA₂, a stable thromboxane A₂ analog, at 37°C for 7 min in the presence or absence of either dexmedetomidine or levomedetomidine with or without yohimbine. STA₂ was used for stimulation in this experiment, because washed platelets cannot be activated by ADP. For the cGMP assay, 100 μl of the platelet suspension containing yohimbine was incubated at 37°C for 3 min in a cylindrical cuvette in the presence or absence of either dexmedetomidine or rilmenigine with or without efaroxan, and then stirred with a magnetic bar at a rate of 1,000 rpm at 37°C for 7 min. In both the cAMP and cGMP assays, the reaction was terminated by adding 10% volume of 0.1 M ice-cold HCl, and the samples were kept at -20°C until measurement.

The cAMP and cGMP measurements were performed using commercially available enzyme immunoassay kits (Cyclic AMP EIA Kit No. 581001 and Cyclic GMP EIA Kit No. 581021 Cayman Chemical, Michigan, USA), according to the manufacturer's protocol.

2.5. Flow cytometry analysis for P-selectin expression on ADP-stimulated and unstimulated platelets

PRP was diluted 10-fold with phosphate-buffered saline (PBS) containing 139 mM NaCl, 8.1 mM NaHPO₄, 1.5 mM KH₂PO₄, and 2.7 mM KCl. For the ADP-stimulated platelet analysis, an aliquot of diluted PRP was incubated with either dexmedetomidine or levomedetomidine in the presence or absence of yohimbine at room temperature for 30 min. Some samples were co-incubated with ADP. For the analysis of unstimulated platelets, an aliquot of diluted PRP was pipetted into a cylindrical cuvette and incubated at 37°C for 3 min with either dexmedetomidine or levomedetomidine in the presence or absence of yohimbine. Then, the samples were stirred constantly with a magnetic bar at a rate of 1,000 rpm at 37°C for 7 min and incubated at room temperature for 30 min without stirring. In both assays, samples were fixed with ice-cold 1% formaldehyde for 60 min on ice and washed twice with ice-cold PBS by centrifugation at 900 *g* for 15 min at 4°C. The pellet was suspended in 100 µl PBS at 4°C. Then, 5 µl of the platelet suspension was co-incubated with PerCP-labeled anti-CD61 antibody and PE-labeled anti-CD62P (P-selectin) antibody in a final volume of 50 µl adjusted with PBS for 60 min at room temperature in the dark. PE-labeled IgG was used to estimate the nonspecific binding. The reaction was stopped by adding ice-cold PBS. Samples were analyzed using a fluorescence-activated cell sorting (FACS) Calibur instrument (Becton Dickinson, San Jose, CA, USA). For each sample, data from 10,000 platelets were collected. Platelets were identified by forward and side scatter intensity and by CD61 expression. P-selectin expression levels on activated platelet surface membranes were recorded as the mean fluorescent intensity (MFI) of PE.

2.6. Statistical Analysis

All data are expressed as mean $\pm$ standard deviation (SD) of three to five separate experiments. The group variances were tested by the Brown-Forsythe test, and statistically equal. All data were compared with one-way analysis of variance (ANOVA), followed by the Dunnett's test. Statistical analyses were performed using Prism 5.0 software (GraphPad Software, San Diego, CA, USA). *P* values less than 0.05 were considered to indicate statistical significance.

3. Results

3.1. Effects of dexmedetomidine on platelet aggregation

Dexmedetomidine (10-500 ng ml⁻¹) enhanced platelet aggregation induced by 0.5 μ M ADP in a dose-dependent manner, while levomedetomidine (500 ng ml⁻¹), an S (-)-enantiomer of medetomidine with an α_2 -adrenoceptor binding affinity much lower than that of dexmedetomidine (Jansson et al., 1994), had no significant effect (Fig. 1A). We next aimed to pharmacologically analyze which receptor is involved in the effect of dexmedetomidine on platelet aggregation. We used 1.5 μ M ADP for stimulation in this experiment, because platelet aggregation induced by 0.5 μ M ADP was too low to examine the effect of antagonists on the enhancing effect. Yohimbine alone did not affect ADP-induced platelet aggregation (data not shown). Fig. 1B shows that the enhancing effect of dexmedetomidine on platelet aggregation was abolished by yohimbine, suggesting that dexmedetomidine activates platelet aggregation through the activation of the α_2 -adrenoceptor in the presence of ADP stimulation. Interestingly, platelet aggregation in the presence of dexmedetomidine and yohimbine was significantly lower than that of the control level, indicating that dexmedetomidine has a suppressive effect on platelet aggregation by a mechanism other than through the α_2 -adrenoceptor. Efaroxan, an I₁-antagonist, abolished the suppressive effect of dexmedetomidine in the presence of yohimbine, but idazoxan, an I₂-antagonist, did not (Fig. 1B). This result suggests that dexmedetomidine suppresses platelet aggregation *via* the I₁-receptor. Methylene blue, a soluble guanylate cyclase (GC) inhibitor, was used to examine whether dexmedetomidine suppresses platelet aggregation by activation of GC. Methylene blue alone did not affect ADP-induced platelet aggregation (data

not shown), but abolished the suppressive effect of dexmedetomidine on ADP-induced platelet aggregation in the presence of yohimbine (Fig. 1B), indicating that GC activation mediates the suppressive effect of dexmedetomidine.

We next examined whether dexmedetomidine affects platelet aggregation in the absence of stimulation. Fig. 1C demonstrates that dexmedetomidine (10 ng ml^{-1}) enhanced spontaneous platelet aggregation, while levomedetomidine (100 ng ml^{-1}) had no significant effect. Furthermore, the enhancing effect of dexmedetomidine was suppressed by yohimbine. Thus, our results show that dexmedetomidine activates platelet aggregation through activation of the α_2 -adrenoceptor regardless of the presence or absence of ADP stimulation.

3.2. Mechanism of α_2 -adrenoceptor-mediated platelet activation

It is well known that a decrease in the cAMP levels plays a major role in the platelet-activating effects of the α_2 -adrenoceptor (Keularts et al., 2000). Fig. 2A shows that dexmedetomidine (10 ng ml^{-1}) suppressed cAMP formation, and this effect of dexmedetomidine was blocked by yohimbine. Levomedetomidine (100 ng ml^{-1}) did not significantly affect cAMP formation with or without yohimbine (Fig. 2B). These results indicate that dexmedetomidine suppresses cAMP formation *via* the α_2 -adrenoceptor, which probably results in the enhancement of platelet aggregation.

Next, we assessed P-selectin expression to analyze the effect of dexmedetomidine on platelet functions other than platelet aggregation, because P-selectin expression increases on the

surface of activated platelets in the process of granule secretion (Furie et al., 2001). Fig. 3 shows that dexmedetomidine (10-100 ng ml⁻¹) enhanced P-selectin expression on both ADP-stimulated and non-stimulated platelet surfaces in a dose-dependent manner, and the enhancing effect was abolished by yohimbine. Levomedetomidine (up to 100 ng ml⁻¹) did not significantly affect surface P-selectin expression (Fig. 3). This result indicates that dexmedetomidine not only enhances platelet aggregation but also increases P-selectin expression *via* the α_2 -adrenoceptor in the presence or absence of ADP stimulation.

3.3. Mechanism of I_1 -receptor-mediated inhibition of platelet aggregation

To elucidate the mechanism for the GC activation involved in the dexmedetomidine-induced inhibition of platelet aggregation mediated by the I_1 -receptor (see above), we tested whether cGMP is increased by dexmedetomidine. Fig. 4 demonstrates that dexmedetomidine (10-100 ng ml⁻¹) increased cGMP levels in a dose-dependent manner in the presence of yohimbine. Rilmenigine (100 μ M), an I_1 -agonist, also increased cGMP levels in the presence of yohimbine. The increase in cGMP production induced by both dexmedetomidine and rilmenigine was abolished by efaroxan. These results suggest that dexmedetomidine increases cGMP levels *via* the I_1 -receptor, leading to GC activation and suppression of platelet aggregation.

4. Discussion

In this study, we aimed to elucidate the effect of dexmedetomidine on human platelet functions *in vitro*. The results demonstrate that dexmedetomidine potentiates and suppresses human platelet function *via* the α_2 -adrenoceptor and the I₁-imidazoline receptor, respectively, as depicted in Fig. 5. This is the first report showing that dexmedetomidine produces a bidirectional effect on human platelet.

Activation of the α_2 -adrenoceptor induces inhibition of adenylyate cyclase (AC) and reduction of cAMP formation from adenosine triphosphate (Limbird, 1988). It has been reported that reduction in cAMP levels induces platelet activation by suppressing cAMP-dependent phosphorylation of the inositol trisphosphate receptor and potentiating calcium release from the endoplasmic reticulum (Keularts et al., 2000). The results in this study showed that dexmedetomidine enhanced ADP-induced platelet aggregation and suppressed platelet cAMP formation *via* the α_2 -adrenoceptor. Together with previous reports, it is suggested that dexmedetomidine activates the α_2 -adrenoceptor leading to suppression of cAMP formation, an increase in cytosolic calcium levels, and finally enhances platelet activation.

Spontaneous platelet aggregation, generally defined as the formation of small-sized aggregates without any stimulation under constant stirring (Ozaki et al., 1994), could be considered as the initial aggregation step leading to thrombus formation. The spontaneously formed small-sized aggregates of platelets play a pivotal role in cardiovascular events and predict the occurrence of these events more reliably than the change in the agonist-induced aggregation (Kajiwara et al., 2001; Miyamoto et al., 2000). On the other hand, P-selectin, one of

the adhesion molecules involved in the interaction between platelets and other cells, stabilizes the initial platelet aggregates in human platelets *in vitro* (Merten and Thiagarajan, 2000), and contributes to the increase and stabilization of arterial thrombi (Christersson et al., 2008; Yokoyama et al., 2005). Clinical studies demonstrated that platelet surface P-selectin expression is increased in the context of arteriosclerosis, ischemic stroke and peripheral arterial disease (Burger and Wagner, 2003; Marquardt et al., 2002; Zeiger et al., 2000). Since our results demonstrated that dexmedetomidine enhances spontaneous aggregation and P-selectin expression, it might be possible that dexmedetomidine enhances thrombus formation particularly in patients with high blood coagulability.

We showed that dexmedetomidine suppressed ADP-induced platelet aggregation *via* the I₁-receptor. The I₁-receptor is expressed in platelets, and neural and epithelial cells (Dahmani et al., 2008; Piletz and Sletten, 1993; Piletz et al., 1996; Savola and Savola, 1996; Virtanen et al., 1988; Wikberg et al., 1991), but the physiological functions are not fully understood. Although signal transduction mechanisms activated by the I₁-receptor remain to be clarified, we hypothesized that cGMP is involved in the I₁-receptor-mediated inhibition of platelet aggregation, because an increase in the intracellular cGMP level can inhibit platelet aggregation (Smolenski, 2012), and clonidine increases cGMP production by the action on the I-receptors in the rat adrenal gland (Regunathan et al., 1990). Our results demonstrated that the suppressive effect of dexmedetomidine on ADP-induced platelet aggregation was reduced by methylene blue, a GC inhibitor, and that cGMP formation was increased by dexmedetomidine, which was inhibited by efaroxan. Thus, it is suggested that dexmedetomidine suppresses ADP-induced

platelet aggregation by I₁-receptor activation resulting in GC activation and an increase in cGMP formation. Further study is necessary to elucidate the mechanism of I₁-receptor-mediated GC activation.

Although there have been several reports on the effects of dexmedetomidine on bleeding during surgery, the results are not consistent and the mechanism for its effect on bleeding has not been fully explored. Durmus et al. (2007) demonstrated that dexmedetomidine decreased bleeding in patients undergoing elective tympanoplasty and septorhinoplasty under general anesthesia. In contrast, Mizrak et al. (2013) reported that dexmedetomidine increased bleeding in pediatric patients undergoing adenotonsillectomy. Our results demonstrated that dexmedetomidine has both enhancing and suppressive effects on platelet functions, and that a drug with α_2 -antagonist-like activity affects which of the two opposite effects becomes evident. Therefore, the effects of dexmedetomidine on bleeding might depend on whether dexmedetomidine is administered together with drugs blocking the α_2 -adrenoreceptor, such as yohimbine, a selective α_2 -antagonist used for treatment of male sexual dysfunction (Tam et al., 2001), and phenoxybenzamine, a non-selective α -antagonist used for perioperative management of pheochromocytoma (Agrawal et al., 2014). The effects of dexmedetomidine on hemostasis might be more apparent in patients whose platelet number in the blood is seriously decreased by a variety of pathophysiological mechanisms. Further studies are necessary to clarify whether dexmedetomidine actually affects platelet functions and hemostasis in the clinical setting.

There are some limitations in this study. The first limitation is that the concentration of dexmedetomidine used in our study was apparently higher than the usual therapeutic

concentration range (0.4-1.2 ng ml⁻¹) (Venn et al., 2002). In fact, Kose et al. (2013) showed that 0.4-1.2 ng ml⁻¹ of dexmedetomidine had no effect on platelet aggregation induced by ADP, collagen, and epinephrine *in vitro*. However, because it was reported that the plasma concentration of dexmedetomidine can reach 10 ng ml⁻¹ in patients with liver dysfunction and the elimination half-life and context-sensitive half-time of dexmedetomidine are prolonged in the elderly and by hypoalbuminemia (Iirola et al., 2012), it might be possible that dexmedetomidine affects platelet functions in the clinical setting. The second limitation is that our study was performed *in vitro* and could not evaluate the effects of blood flow, blood vessels, shear stress on platelets, and the interactions between platelets and other blood cells. To clarify the involvement of these factors in the effect of dexmedetomidine on hemostasis, an *in vivo* study is required. The third limitation is that we analyzed the effect of dexmedetomidine on platelet aggregation induced by only ADP. Because we cannot exclude the possibility that the effect of dexmedetomidine is dependent on the type of stimulation, we should examine the effect of dexmedetomidine on platelets stimulated with other agonists including collagen and thrombin in future.

5. Conclusions

The present study demonstrated that dexmedetomidine has both enhancing and suppressive effects on platelet functions *in vitro*. Our data suggest that the enhancing effect is mediated by activation of the α_2 -adrenoceptor and inhibition of AC, while the suppressive effect is mediated by activation of the I₁-receptor and GC.

Authors' Contributions

S.K.: data collection, data analysis, and drafting of the paper. N.S.: help with data collection and analysis. H.H.: study conception and design, interpretation of data, and revision of the manuscript. K.F.: supervising the study and revision of the manuscript. All authors have approved the final version of the paper.

Declaration of interest

None declared.

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Figure legends

Fig. 1. Effects of dexmedetomidine on human platelet aggregation. (A) Effects of dexmedetomidine (DEX; 1-500 ng ml⁻¹) and levomedetomidine (LEVO; 500 ng ml⁻¹) on platelet aggregation induced by 0.5 μ M ADP. The data are expressed as mean $\pm$ SD (n = 5). **P* < 0.05 vs. DEX (-), LEVO (-). (B) Effects of dexmedetomidine (DEX; 10 ng ml⁻¹) on platelet aggregation induced by 1.5 μ M ADP in the presence and absence of yohimbine (YH; 10 μ M), efaroan (EX; 10 μ M), idazoxan (IX; 10 μ M), and methylene blue (MB; 3 μ M). The data are expressed as mean $\pm$ SD (n = 5). **P* < 0.05 vs. DEX (-), YH (-), EX(-), IX (-), MB (-). (C) Effects of dexmedetomidine (DEX; 1-10 ng ml⁻¹) and levomedetomidine (LEVO; 100 ng ml⁻¹) on spontaneous platelet aggregation in the absence and presence of yohimbine (YH; 10 μ M). The data are expressed as mean $\pm$ SD (n = 5). **P* < 0.05 vs. DEX (-), YH (-), LEVO (-). Dexmedetomidine enhanced both ADP-induced and spontaneous platelet aggregation, whereas levomedetomidine showed no effect on platelet aggregation. Dexmedetomidine suppressed ADP-induced platelet aggregation in the presence of yohimbine, and this effect was abolished by efaroan and methylene blue.

Fig. 2. Effects of dexmedetomidine (DEX; 10 ng ml⁻¹) (A) and levomedetomidine (LEVO; 100 ng ml⁻¹) (B) on cAMP formation in platelets stimulated with 0.1 μ M 9, 11-epithio-11, 12-methanohromboxane A₂ (STA₂) for 7 min in the presence and absence of yohimbine (YH; 100 μ M). The data are expressed as mean $\pm$ SD (n = 3). **P* < 0.05 vs. DEX (-), YH (-). Dexmedetomidine suppressed cAMP formation, and this effect was blocked by yohimbine,

whereas levomedetomidine did not significantly affect cAMP formation in the presence or absence of yohimbine.

Fig. 3. Effect of dexmedetomidine (DEX; 1-100 ng ml⁻¹) and levomedetomidine (LEVO; 100 ng ml⁻¹) on surface P-selectin expression in platelets. Experiments were performed with (A) or without (B) stimulation by 10 μM ADP in the presence and absence of yohimbine (YH; 10 μM). MFI, mean fluorescence intensity. The data are expressed as mean ± SD (n = 4). **P* < 0.05 vs. DEX (-), YH (-), LEVO (-). In the presence or absence of ADP stimulation, dexmedetomidine enhanced P-selectin expression in a yohimbine-sensitive manner, whereas levomedetomidine did not significantly affect P-selectin expression.

Fig. 4. Effect of dexmedetomidine (DEX; 1-100 ng ml⁻¹) and rilmenigine (RLM; 100 μM) on cGMP formation in the presence of yohimbine (YH; 100 μM). Efaroxan (EX; 10 μM) abolished the enhancing effect of dexmedetomidine and rilmenigine. The data are expressed as mean ± SD (n = 5). **P* < 0.05 vs. DEX (-), RLM (-), EX (-).

Fig. 5. Bidirectional effects of dexmedetomidine (DEX) on platelet aggregation. DEX activates the α₂-adrenoceptor (α₂R), leading to suppression of adenylate cyclase and cAMP formation, and finally enhances platelet aggregation. On the other hand, DEX activates guanylate cyclase and increases cGMP levels *via* the I₁-imidazoline receptor (I₁R), leading to suppression of platelet

aggregation. White and black arrows represent the DEX-induced effects involving α_2 R and I_1 R, respectively. Upward and downward arrows show activation/increase and inhibition/decrease, respectively. The interrupted lines represent the effects of the inhibitors used in this study.

Yohimbine and efaroxan inhibit the DEX-induced α_2 - and I_1 -activation, respectively. Methylene blue suppresses guanylate cyclase activity. The dotted line represents the action of rilmenidine, which activates I_1 R leading to increase in cGMP. ATP; adenosine triphosphate, GTP; guanosine triphosphate, cAMP; cyclic adenosine monophosphate, cGMP; cyclic guanosine monophosphate.

Professor F.P. Nijkamp,
Editor-in-Chief, European Journal of Pharmacology.

Dear Dr. Nijkamp:

On behalf of my co-authors, I would like to ask you to consider our manuscript entitled "Bidirectional effects of dexmedetomidine on human platelet functions *in vitro*" for publication in the *European Journal of Pharmacology* as an original research report.

Because platelets are essential for keeping hemostasis, information on the effects of perioperatively used drugs including anesthetics on platelet functions is indispensable for perioperative patient care. This is the first report showing both enhancing and suppressive effects of dexmedetomidine on human platelet functions *in vitro*. Our results suggest that the enhancing effect is mediated by activation of the α_2 -adrenoceptor and inhibition of adenylate cyclase, while the suppressive effect is mediated by activation of the imidazoline I₁-receptor and guanylate cyclase. Because dexmedetomidine is frequently used for sedation and analgesia for critically ill patients, our results would provide useful information for intensive care. We feel that the findings from this study will be of particular interest to the readers of the *European Journal of Pharmacology*.

This manuscript has not been published and is not under consideration for publication elsewhere. All the authors have read the manuscript and have approved this submission. Financial support for this study was provided solely by departmental funds. The authors report no conflicts of interest.

I hope you find our manuscript worthy of publication in the *European Journal of Pharmacology*.
I am looking forward to hearing from you.

Sincerely,

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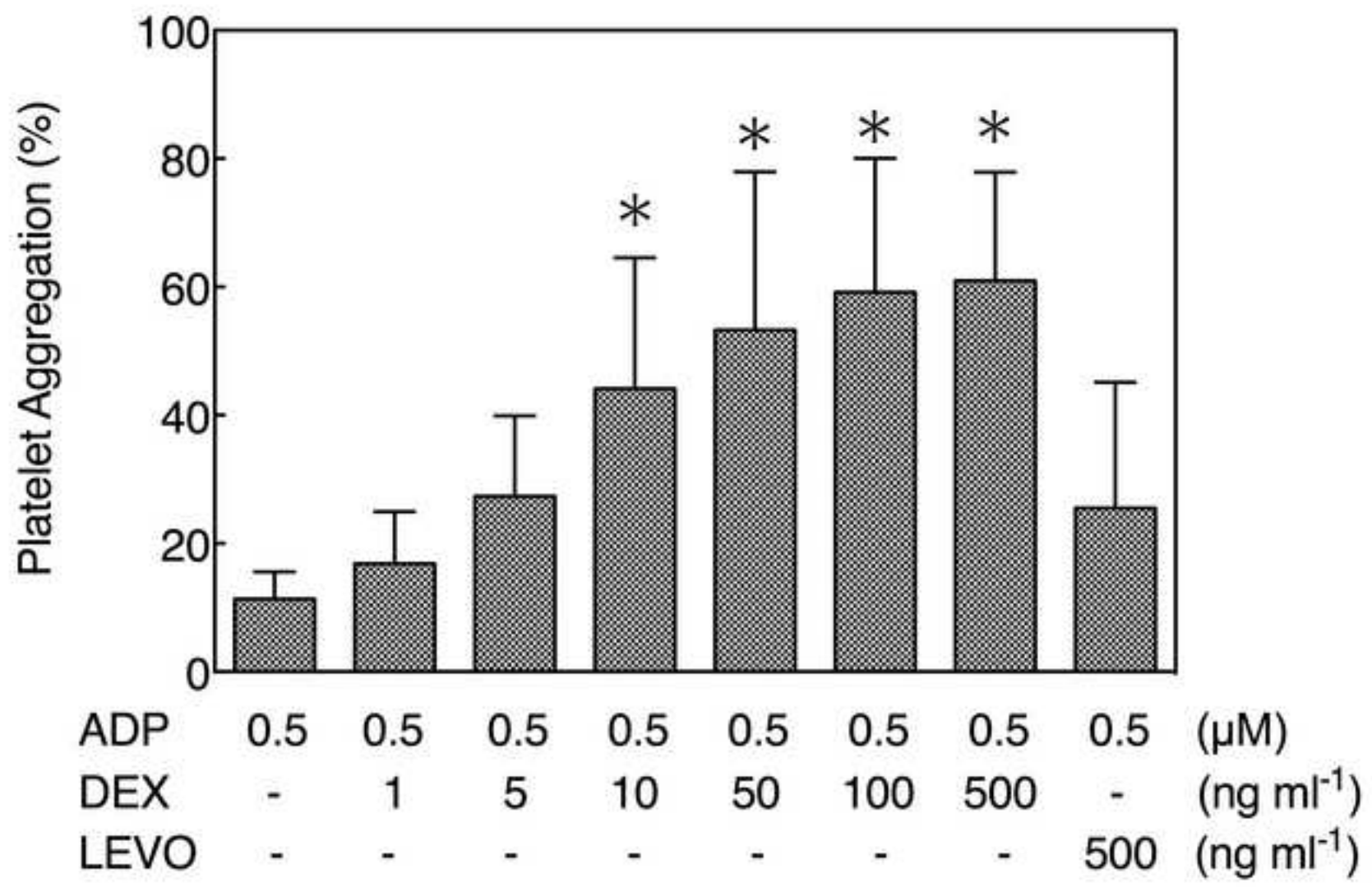


Fig. 1A

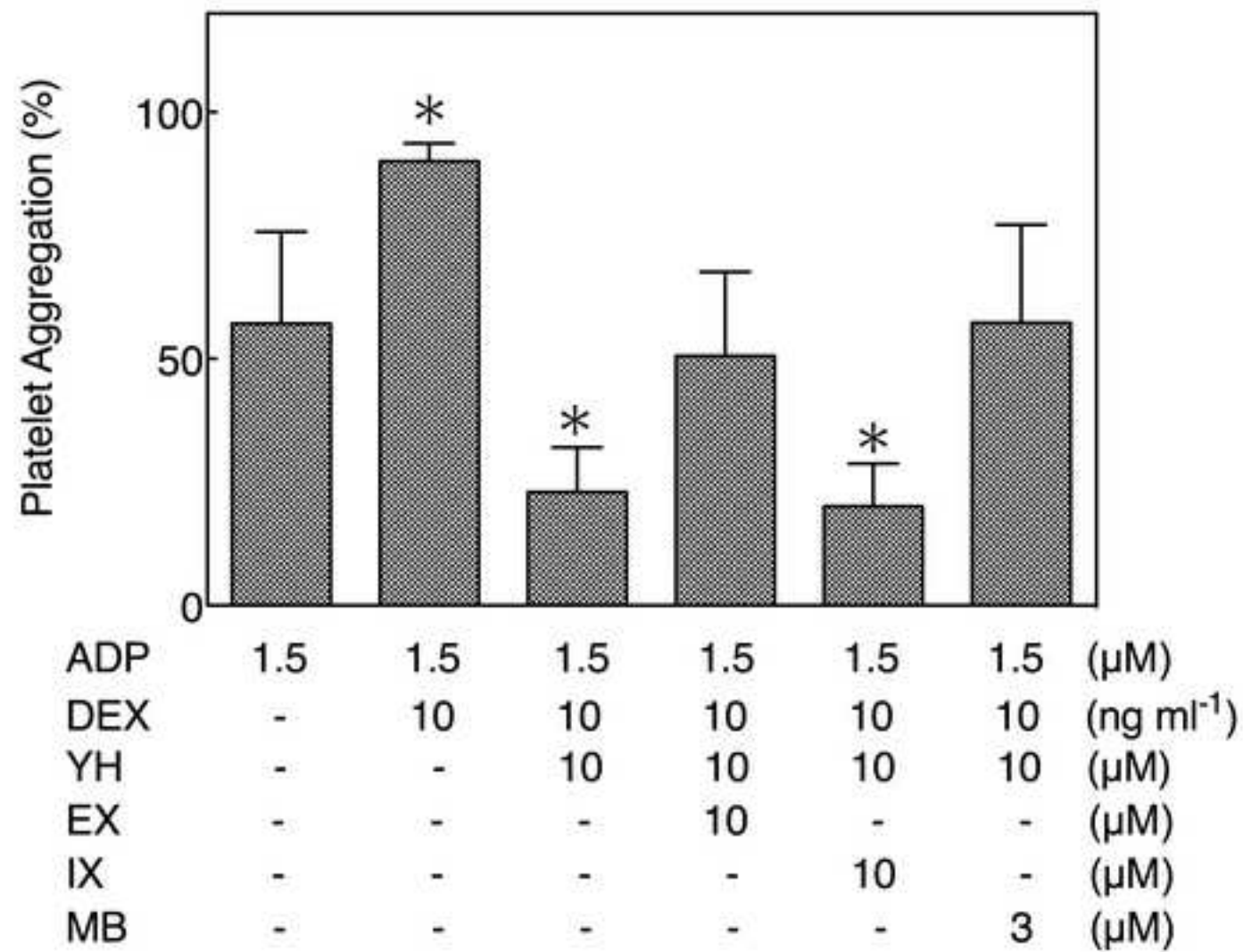


Fig. 1B

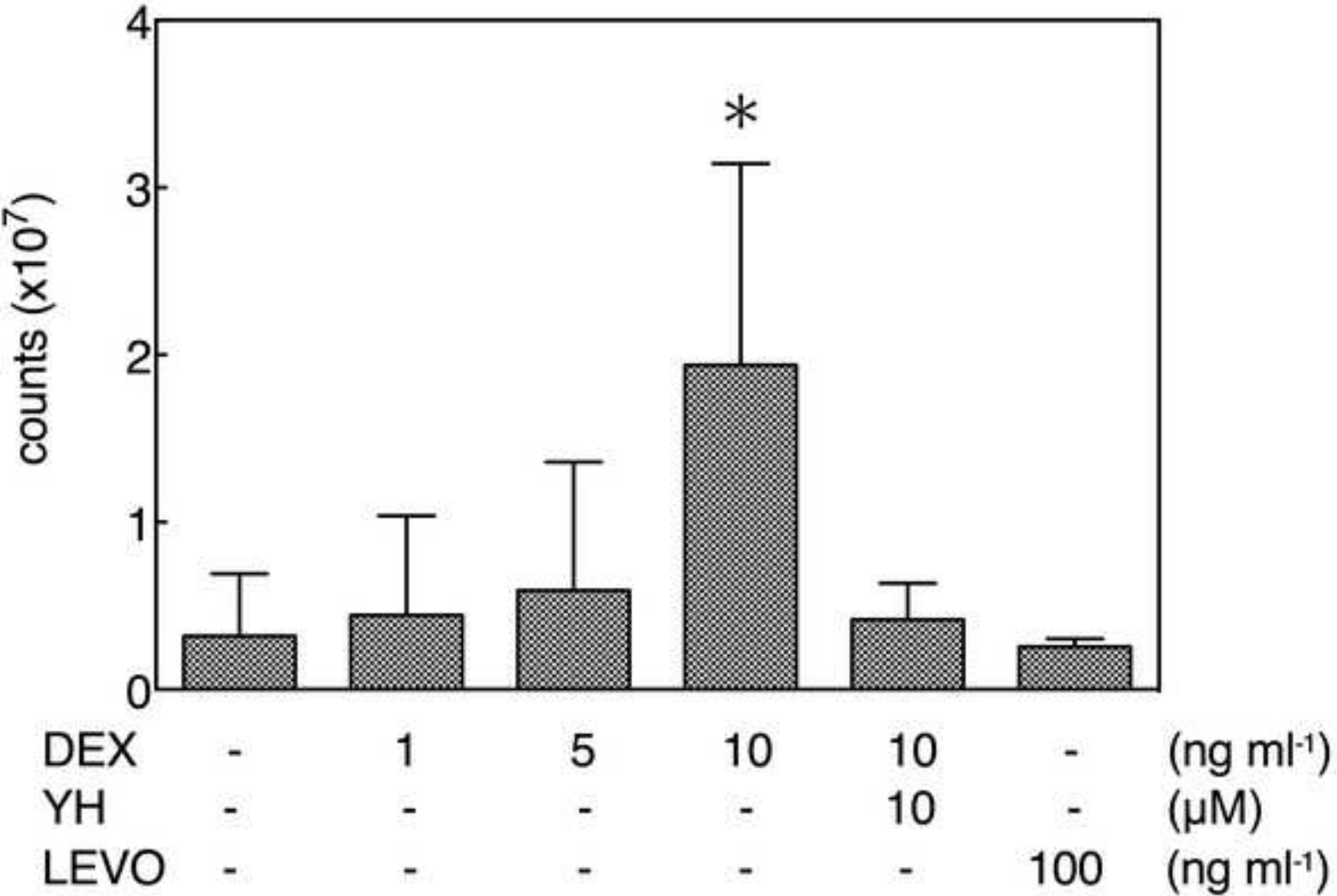


Fig. 1C

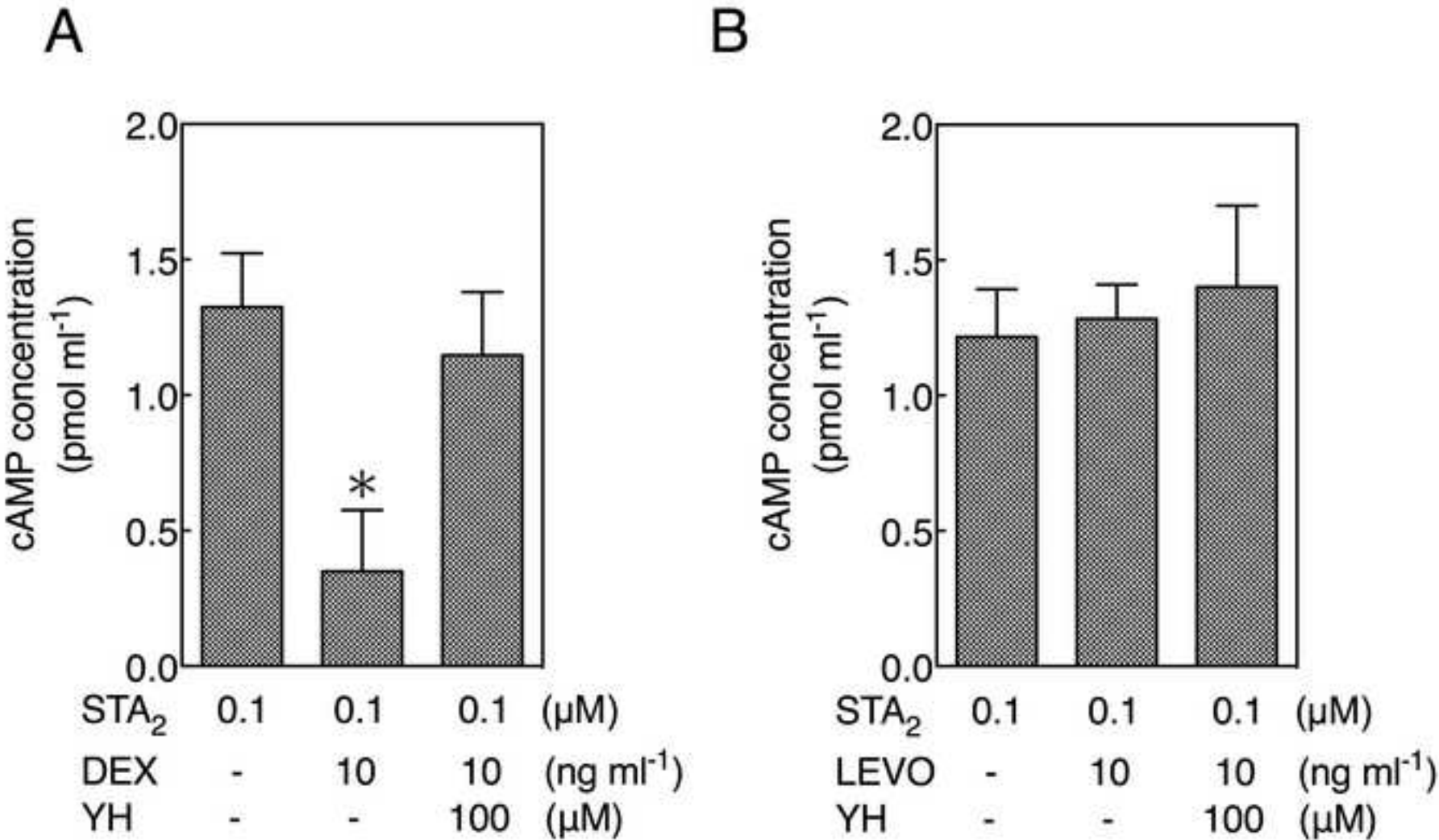
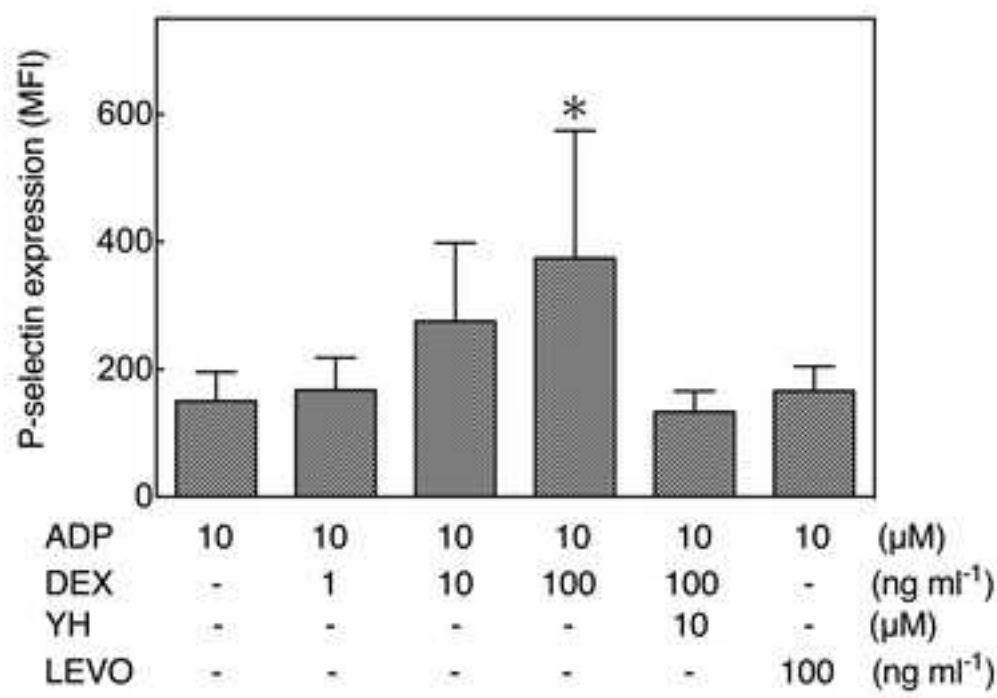


Fig. 2

A



B

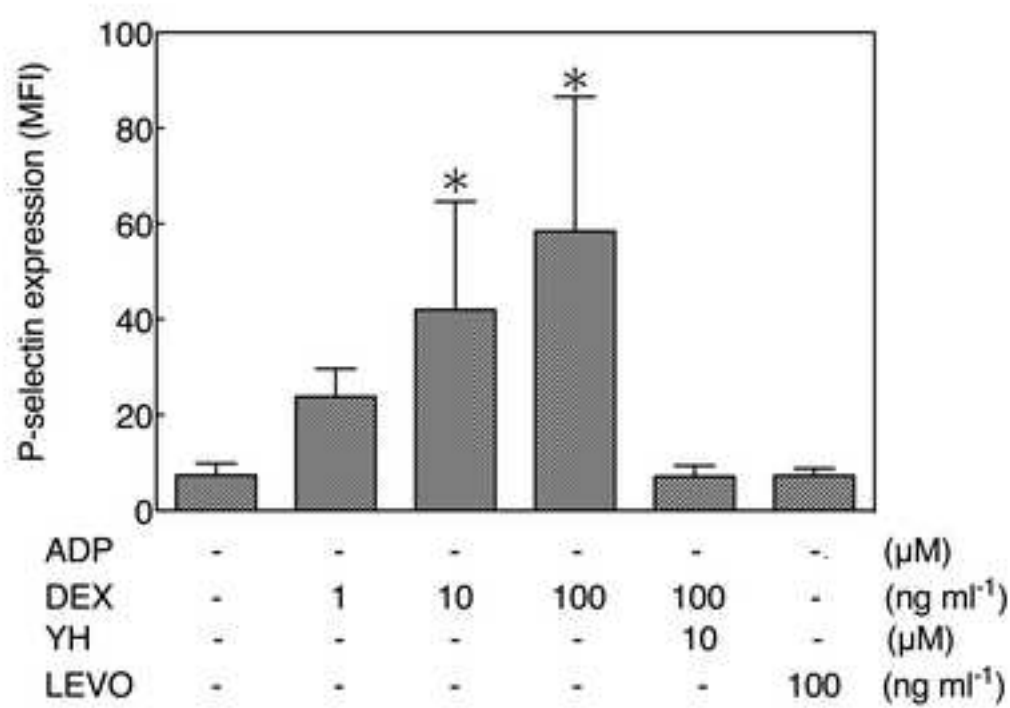


Fig. 3

Figure4
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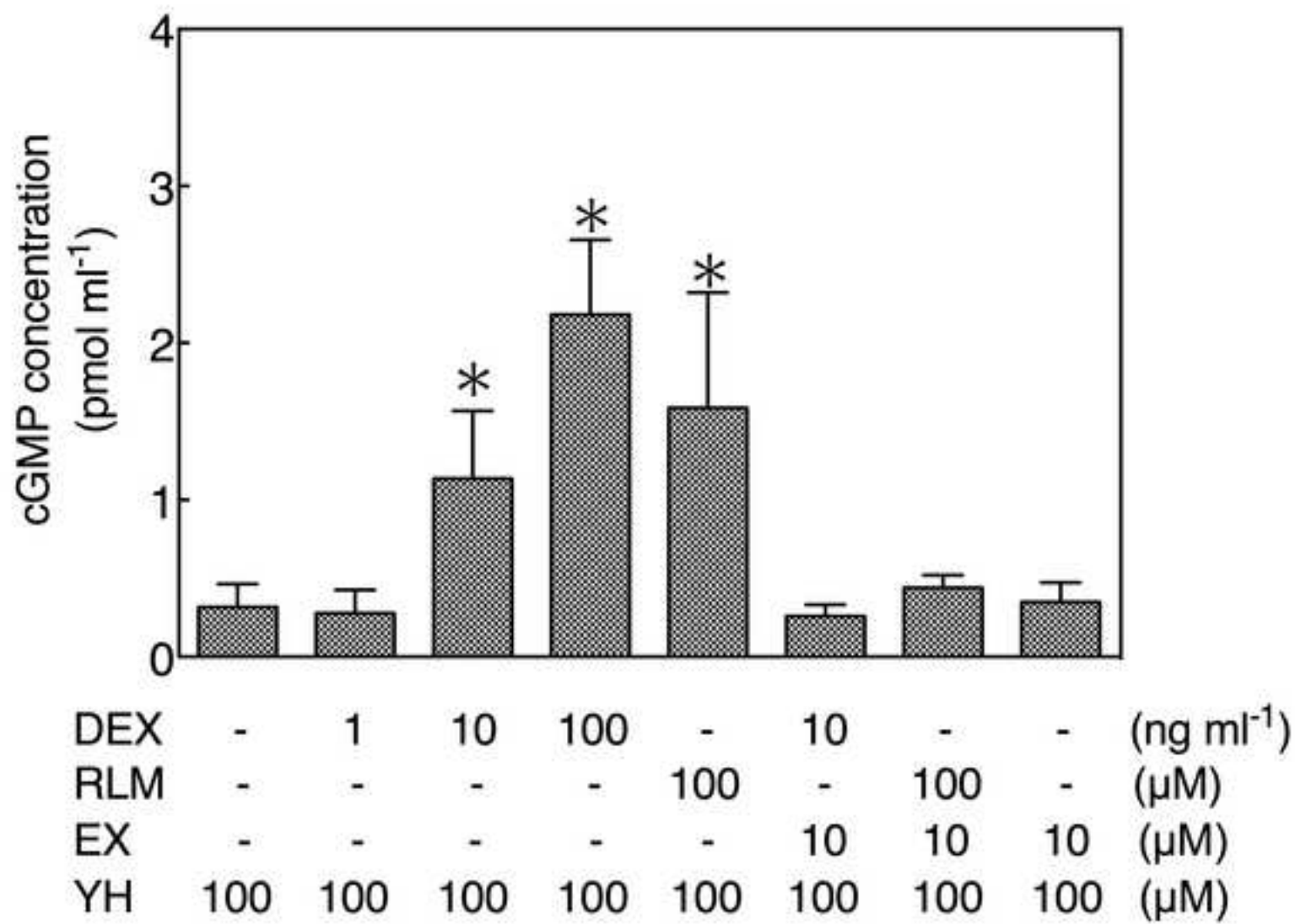


Fig. 4

Figure5

