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

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ABSTRACT

Platelets express the imidazoline (I)-receptor, I_1 and I_2, 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. We showed that dexmedetomidine enhances platelet aggregation. But in the presence of yohimbine, an α_2-antagonist, dexmedetomidine suppressed platelet aggregation. Efaroxan, an I_1-antagonist, and methylene blue, a soluble guanylate cyclase inhibitor, abolished the suppressive effect of dexmedetomidine, whereas idazoxan, an I_2-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 I_1-imidazoline receptor, respectively.

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, 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 S (+)-enantiomer of medetomidine, (S)-4-[1-(2,3-Dimethylphenyl)ethyl]-1H-imidazole 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., 1997; Ernsberger and Haxhiu, 1997; 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

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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-imidazoline 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 R (-)-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 A_2 (STA_2) 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 assessed by comparing small aggregate formation without agonist stimulation using an ultrasensitive platelet aggregation analyzer (PA-200, Kowa, Tokyo, Japan). Total scattered light intensity detected during 10-s period from particles with each scattered light intensity of 25–400 mV was recorded, and the area under the curve for 7 min of sample stirring was used as index of small aggregate (9–25 μm) formation (Ozaki et al., 1994).

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

Washed platelets were used in 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 Na_2HPO_4, 2 mM NaH_2PO_4, 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 10^6 platelets μl^{-1} or 2.5×10^6 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 Na_2HPO_4, 145 mM NaCl, 5 mM 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 1000 rpm at 37 °C. Platelets were stimulated with 0.1 μM STA_2, a stable thromboxane A_2 analog, at 37 °C for 7 min in the presence or absence of either dexmedetomidine or levomedetomidine with or without yohimbine. STA_2 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 1000 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 $NaHPO_4$, 1.5 mM KH_2PO_4, 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 1000 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