Effects of imidazoline and nonimidazoline α-adrenoceptor agonists and antagonists on platelet aggregation in cats

（ネコの血小板凝集能に及ぼすイミダゾリンおよび非イミダゾリンα-アドレナリン受容体作動薬と遮断薬の効果）

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General Introduction

Mammalian platelets vary widely in their responses to catecholamines and other α-adrenergic agents.[29,60,66] In humans, adrenaline- and noradrenaline-induced platelet aggregation is mediated by α_2-adrenoceptors; this aggregation is blocked by α_2-adrenoceptor antagonists but not by α_1-adrenoceptor antagonists.[20,26,32,53] Radioligand binding studies have revealed the existence of α_2-adrenoceptors on platelet membranes of dogs, cats, rabbits,[24] and humans.[31,40,41,59] However, there is a lack of α_2-adrenoceptors on platelet membranes of rats, cattle, and horses.[24] Adrenaline is considered a rather weak platelet agonist, the function of which is primarily to sensitize platelets to other activating agents in humans.[3,25] In dogs,[23] cats,[22,69] and rabbits,[75] adrenaline alone does not induce platelet aggregation but it does potentiate platelet aggregation stimulated by other platelet agonists including adenosine diphosphate (ADP), collagen, and thrombin. This adrenaline-potentiated platelet aggregation is also mediated by α_2-adrenoceptors in dogs[23] and rabbits.[75] In contrast to humans, dogs, and rabbits, adrenaline in cattle and horses does not potentiate platelet aggregation induced by other platelet agonists such as ADP, collagen, thrombin, or platelet-activating factor.[7,61,64,76]

Some adrenoceptor agents have imidazoline-like chemical structures. Clonidine, an imidazole α_2-adrenoceptor agonist, has a complex effect on platelets. For example, clonidine binds with a high affinity to α_2-adrenoceptors on platelets but induces only limited platelet aggregation, in contrast to the effect of endogenous agonists such as adrenaline.[20,26] Moreover, clonidine potentiates ADP-induced aggregation of human platelets but has inhibitory activity for adrenaline- and noradrenaline-induced platelet aggregation.[59,63] Although the mechanism underlying these conflicting actions of clonidine is unclear, imidazoline agents may interact with

non–α_2-adrenoceptor binding sites on platelets.[5,11,12] Nonadrenergic Imidazoline-preferring binding site 1(I_1) and Imidazoline-preferring binding site 2(I_2) receptors that are pharmacologically distinct from α_2-adrenoceptors have been detected in human,[39,51,52,78] canine, feline, bovine, and equine platelets.[24] Furthermore, canine, feline, bovine, and equine platelets have I_1 receptors that are defined by binding to tritiated clonidine, but murine and leporine platelets do not have I_1 receptors. Conversely, platelets of all species have I_2 receptors that are defined by binding to tritiated idazoxan.[24] In addition, the density of I_1 and I_2 receptors and α_2-adrenoceptors differs among animal species.[24] These variations for receptors may reflect differences among animal species regarding the platelet aggregation response. However, there is no information available concerning the platelet aggregatory effects of α-adrenergic agents in cats. Comparative studies on the effects of imidazolines on aggregation of feline platelets may be important for the characterization of platelet receptors and may be useful to elucidate the function of imidazoline receptors.

In cats, α_2-adrenoceptor agonists such as xylazine, medetomidine, and dexmedetomidine are widely used as sedative, analgesic, and muscle relaxant agents, whereas α_2-adrenoceptor antagonists such as atipamezole and yohimbine are often used for to reverse the effects of the aforementioned agonists. These drugs differ in that medetomidine, dexmedetomidine, and atipamezole have imidazoline-like chemical structures, whereas xylazine and yohimbine do not. It also may be important to determine whether imidazoline structures affect the response of platelets.

In cats, overactivity of the sympathetic nervous system and increased catecholamine concentrations are induced in conditions such as pheochromocytoma[72] endotoxin shock[17] and acute stress[56]. Hypercatecholaminemia reportedly has an influence on hemostasis such as

disseminated intravascular coagulation[6] and thromboembolism[10,14,62] by acting on platelets. Because both medetomidine and xylazine reduce plasma concentrations of adrenaline and noradrenaline in cats[27] it may be important on blood homeostasis to examine the platelet response in cats administered systemically with medetomidine or xylazine. However, to the best of our knowledge, there are no published reports on the blood platelet aggregation in cats that were administered xylazine or medetomidine systemically.

Therefore, this study was conducted to investigate the effects of imidazoline and nonimidazoline α-adrenoceptor agonists and antagonists including medetomidine, dexmedetomidine, xylazine, atipamezole, and yohimbine on platelet aggregation in cats. In chapter 1, the study was aimed to investigate the effects of various imidazoline or nonimidazoline α-adrenergic agents on *in vitro* platelet aggregation and antiaggregation in healthy cats. In chapter 2, the study aimed to investigate and compare the effects of medetomidine and xylazine administered systemically on *ex vivo* platelet aggregation in healthy cats.

Chapter 1

Effects of imidazoline and non-imidazoline α-adrenoceptor agonists and antagonists, including xylazine, medetomidine, dexmedetomidine, yohimbine, and atipamezole, on aggregation of feline platelets

Introduction

Platelet responses to catecholamines and other α-adrenergic agents differ widely among animal species.[29,60,66] In humans, adrenaline- and noradrenaline-induced platelet aggregation is mediated by α_2-adrenoceptors; this aggregation is blocked by α_2-adrenoceptor antagonists but not by α_1-adrenoceptor antagonists.[20,26,32,53] Radioligand binding studies have revealed the existence of α_2-adrenoceptors on platelet membranes of dogs, cats, rabbits,[24] and humans;[31,40,41,59] however, there is a lack of α_2-adrenoceptors on platelet membranes of rats, cattle, and horses.[24] Adrenaline is considered a rather weak platelet agonist, the function of which is primarily to sensitize platelets to other activating agents in humans.[3,25] Adrenaline alone does not induce platelet aggregation in dogs,[23] cats,[22,69] and rabbits,[75] but it does potentiate platelet aggregation stimulated by other platelet agonists including ADP, collagen, and thrombin. Adrenaline-potentiated platelet aggregation is also mediated by α_2-adrenoceptors in dogs[23] and rabbits.[75] Conversely, in contrast to humans, dogs, and rabbits, adrenaline in cattle and horses does not potentiate platelet aggregation induced by other platelet agonists such as ADP, collagen, thrombin, or platelet-activating factor.[7,61,64,76] Physiologic concentrations of adrenaline enhance shear-dependent platelet aggregation and platelet-to-platelet interactions on collagen.[19,45]

Overactivity of the sympathetic nervous system and increased catecholamine concentrations are induced in conditions such as pheochromocytoma,[72] endotoxin shock,[16,21] and acute stress[56] in cats. Hypercatecholaminemia reportedly has an influence on hemostasis (eg, disseminated intravascular coagulation[6] and thromboembolism[10,13,62]) through actions on platelets. Information about drugs that inhibit platelet aggregation stimulated by catecholamines may also be useful for

the control and management of hemostasis in diseases or conditions associated with hypercatecholaminemia in small animals.

The imidazoline chemical structure is found in many pharmaceutical drugs with a variety of biological activities, including antifungal (eg, miconazole), antihypertensive (eg, losartan), antiulcer (eg, cimetidine), and antiplatelet agents that inhibit thromboxane A2 synthesis (eg, ozagrel). Some adrenoceptor agents have imidazoline-like chemical structures. Clonidine, an imidazole α_2-adrenoceptor agonist, has a complex effect on platelets. For example, clonidine binds with a high affinity to α_2-adrenoceptors on platelets but induces only limited platelet aggregation, in contrast to the effect of endogenous agonists such as adrenaline.[20,26] Moreover, clonidine potentiates ADP-induced aggregation of human platelets but has inhibitory activity for adrenaline- and noradrenaline-induced platelet aggregation.[59,63] Although the mechanism underlying these conflicting actions of clonidine is unclear, imidazoline agents may interact with non–α_2-adrenoceptor binding sites on platelets.[5,11,12] Two clonidine-related drugs reportedly inhibit platelet adenylate cyclase through non–α_2-adrenoceptor mechanisms because their effects are not blocked by yohimbine.[16] In addition, a clonidine-displacing substance extracted from bovine brain tissue[4] is recognized as a noncatecholamine endogenous ligand and interacts with nonadrenoceptor sites in the brainstem, which was determined via the use of tritiated *p*-aminoclonidine.[38] Nonadrenergic I_1 and I_2 receptors that are pharmacologically distinct from α_2-adrenoceptors have been detected in human,[39,51,52,78] canine, feline, bovine, and equine platelets.[24] Furthermore, canine, feline, bovine, and equine platelets have I_1 receptors that are defined by binding to tritiated clonidine, but murine and leporine platelets do not have I_1 receptors. Conversely, platelets of all species have I_2 receptors that are defined by binding to tritiated idazoxan.[24] In addition, the density of I_1 and I_2 receptors and α_2-adrenoceptors differs

among animal species.[24] These variations for receptors may reflect differences among animal species regarding the platelet aggregation response. However, there is no information available concerning the platelet aggregatory effects of α-adrenergic agents in cats. Comparative studies on the effects of imidazolines on aggregation of feline platelets may be important for the characterization of platelet receptors and may be useful to elucidate the function of imidazoline receptors.

In cats, α_2-adrenoceptor agonists such as xylazine, medetomidine, and dexmedetomidine are widely used as sedative, analgesic, and muscle relaxant agents, whereas α_2-adrenoceptor antagonists such as atipamezole and yohimbine are often used for to reverse the effects of the aforementioned agonists. These drugs differ in that medetomidine, dexmedetomidine, and atipamezole have imidazoline-like chemical structures, whereas xylazine and yohimbine do not. It also may be important to determine whether imidazoline structures affect the response of platelets. Therefore, the objective of the study reported here was to investigate effects of various imidazoline or nonimidazoline α-adrenergic agents on in vitro aggregation and antiaggregation of feline platelets.

Materials and methods

Sample

Blood was collected from 12 healthy adult mixed-breed cats. Cats were from 2 to 7 years of age and comprised 8 males and 4 females; body weight ranged from 3.2 to 5.0 kg. Cats were housed in a laboratory with appropriate animal management facilities and fed a standard commercial dry food; water was available ad libitum. Blood was repeatedly collected from each cat at intervals of ≥ 2 weeks. Cats were examined (physical examination and hematologic

analysis) prior to each blood collection to ensure that they were healthy. The study protocol was approved by the Animal Research Committee of Tottori University.

Preparation of citrated platelet plasma

Blood was collected for use in platelet aggregation experiments. Food was withheld from cats for at least 6 hours before blood collection. Jugular blood samples (9 mL) were collected with a 21-gauge needle into a 10-mL plastic syringe containing 3.8% sodium citrate solution (ratio, 1 part anticoagulant to 9 parts blood). Citrated platelet plasma was prepared in accordance with a modification of methods described elsewhere.[22,23] Blood was centrifuged at 90 to 110 × *g* for 10 to 15 minutes to obtain Platelet-rich plasma (PRP). The Platelet-poor plasma (PPP) then was obtained by centrifuging PRP at 1,500 × *g* for 15 minutes. The final platelet count was adjusted to 25 to 30 × 10^4 platelets/μL via dilution with autologous PPP.

Aggregation experiments

The study consisted of 7 platelet aggregation experiments; aggregation experiments were performed as previously described.[23,26,75,76] Briefly, a turbidimetric method was used. An aliquot (200 μL) of PRP was placed in an aggregometer (MCM Hema tracer 804, LMS Co Ltd, Tokyo, Japan) at 37°C, and an aliquot (22 μL) of test agent was added to the PRP 1 minute later. The percentage aggregation was standardized via the assumption that PPP and PRP represented 100% and 0% light transmission, respectively.

Drugs used in the study included L-adrenaline, L-noradrenaline, and phenoxybenzamine HCl (Tokyo Kasei Industries Co, Tokyo, Japan); *p*-aminoclonidine HCl, antazoline HCl, clonidine HCl, idazoxan HCl, methoxamine HCl, moxonidine HCl, naphazoline HCl, oxymetazoline HCl,

phentolamine HCl, L-phenylephrine HCl, prazosin HCl, tolazoline HCl, xylazine HCl, xylometazoline HCl, and yohimbine HCl (Sigma Chemical Co, St Louis, Mo); tramazoline HCl (Boehringer-Ingelheim Corp, Hyogo, Japan); atipamezole HCl, detomidine HCl, and medetomidine HCl (Farmos Group Ltd, Turku, Finland); dexmedetomidine HCl (Maruishi Pharmaceutical Co Ltd, Osaka, Japan); and ADP and collagen (LMS Co Ltd, Tokyo, Japan).

Adrenaline and noradrenaline were dissolved in 0.04M HCl solution and then diluted with sterile saline (0.9% NaCl) solution. Prazosin and phenoxybenzamine were dissolved in sterile distilled water and then diluted with sterile saline solution. All other drugs were dissolved in sterile saline solution. In addition, sterile saline solution was used as a negative control agent throughout the experiments.

Both prazosin and phenoxybenzamine could be dissolved in sterile distilled water at concentrations up to 100 μmol/L, but both agents at higher concentrations (1 mmol/L) became cloudy and could not be completely dissolved in distilled water. Because cloudy solutions influence the percentage aggregation on the basis of light transmission, we did not determine percentage aggregation of both agents at 1 mmol/L. In addition, we did not determine percent aggregation of higher concentrations of 1 mmol/L medetomidine and 0.1 to 1 mmol/L dexmedetomidine, because we used the drug solution products rather than drug powders for both agents.

The drugs were categorized as α-adrenoceptor agonists (adrenaline, noradrenaline, clonidine, *p*-aminoclonidine, xylazine, medetomidine, detomidine, dexmedetomidine, oxymetazoline, xylometazoline, moxonidine, tramazoline, naphazoline, phenylephrine, and methoxamine), α-adrenoceptor antagonists (yohimbine, phentolamine, atipamezole, idazoxan, tolazoline, phenoxybenzamine, and prazosin), imidazoline α-adrenoceptor agonists (clonidine, *p*-

aminoclonidine, medetomidine, detomidine, dexmedetomidine, oxymetazoline, xylometazoline, moxonidine, tramazoline, and naphazoline), imidazoline α-adrenoceptor antagonists (phentolamine, atipamezole, idazoxan, and tolazoline), nonimidazoline α-adrenoceptor agonists (adrenaline, noradrenaline, xylazine, phenylephrine, and methoxamine), nonimidazoline α-adrenoceptor antagonists (yohimbine, phenoxybenzamine, and prazosin), and imidazoline non–α-adrenoceptor agonists (antazoline).

The protocol of each experiment is summarized in Figure 1. In experiment 1, platelet aggregation effects of α-adrenergic agents alone were evaluated. An aliquot of PRP was placed in the aggregometer; 1 minute later, 21 α-adrenoceptor agonists or antagonists (Adrenaline, noradrenaline, clonidine, xylazine, medetomidine, detomidine, dexmedetomidine, oxymetazoline, xylometazoline, moxonidine, tramazoline, naphazoline, phenylephrine, methoxamine, antazoline, yohimbine, phentolamine, atipamezole, idazoxan, tolazoline, and prazosin) at final concentrations of 0.1 nmol/L to 1 mmol/L (except for medetomidine at 1 mmol/L, dexmedetomidine at 0.1 to 1 mmol/L, and prazosin at 1 mmol/L) were added to the PRP (time 0), and the maximum percentage aggregation was recorded during the subsequent 10-minute interval.

In experiment 2, aggregation effects of ADP or collagen were examined. An aliquot of PRP was placed in the aggregometer; 1 minute later, ADP (0 to 10 μmol/L) or collagen (0 to 10 μg/mL) was added to the PRP (time 0), and the maximum percentage aggregation was recorded during the subsequent 10-minute interval.

In experiment 3, the stimulatory or inhibitory effects of α-adrenergic agents on ADP- or collagen-induced aggregation were examined. An aliquot of PRP was placed in the aggregometer; 1 minute later, 12 α-adrenergic agents (adrenaline, noradrenaline, oxymetazoline,

xylometazoline, clonidine, medetomidine, detomidine, xylazine, naphazoline, tramazoline, phenylephrine, and methoxamine) at final concentrations of 1 nmol/L to 1 mmol/L, except for medetomidine at 1 mmol/L, were added and 1 minute after that, ADP (0.5 μmol/L) or collagen (0.5 or 1 μg/mL) was added (time 0). In addition, dose-dependent effects of ADP on platelet aggregation were evaluated. Adrenaline (100 μmol/L), noradrenaline (100 μmol/L), oxymetazoline (10 μmol/L), or xylometazoline (10 μmol/L) were added to PRP; 1 minute later, ADP (0.1 to 10 μmol/L) was added (time 0). The maximum percentage aggregation during the 10-minute interval after the addition of ADP or collagen was recorded.

In experiment 4, inhibitory effects of imidazoline and nonimidazoline α-adrenergic agents on platelet aggregation induced by adrenaline and ADP were examined. An aliquot of PRP was placed in the aggregometer; 1 minute later, 19 agents (yohimbine, atipamezole, idazoxan, phentolamine, tolazoline, phenoxybenzamine, prazosin, naphazoline, tramazoline, xylometazoline, antazoline, clonidine, medetomidine, oxymetazoline, detomidine, *p*-aminoclonidine, phenylephrine, xylazine, and methoxamine) at final concentrations of 1 nmol/L to 1 mmol/L (except for medetomidine, phenoxybenzamine, and prazosin at 1 mmol/L) were added. Then 0.5 minutes later, adrenaline (100 μmol/L) was added, and 0.5 minutes after that, ADP (1 μmol/L) was added (time 0). The maximum percentage aggregation was determined during the 10-minute interval after the addition of ADP.

In experiment 5, inhibitory effects of imidazoline and nonimidazoline α-adrenergic agents on platelet aggregation induced by adrenaline and collagen were examined. An aliquot of PRP was placed in the aggregometer; 1 minute later, 14 agents (yohimbine, atipamezole, idazoxan, phentolamine, tolazoline, prazosin, naphazoline, antazoline, clonidine, medetomidine, oxymetazoline, dexmedetomidine, moxonidine, and xylazine) at final concentrations of 1 nmol/L

to 100 μmol/L, except for dexmedetomidine at 100 μmol/L, were added. Adrenaline (10 μmol/L) was added 0.5 minute later, and collagen (1, 2, or 3 μg/mL) was added 0.5 minutes after that (time 0). The maximum percentage aggregation was determined during the 10-minute interval after the addition of collagen.

In experiment 6, effects of a combination of α_2-adrenoceptor agonists and antagonists on platelet aggregation induced by adrenaline and collagen were examined. An aliquot of PRP was placed in the aggregometer; 1 minute later, 2 agonists (yohimbine and atipamezole), 2 antagonists (xylazine and medetomidine), and their combinations were added (final concentrations, 1 nmol/L to 100 μmol/L). Adrenaline (10 μmol/L) was added 0.5 minutes later, and collagen (1, 2, or 3 μg/mL) was added 0.5 minutes after that (time 0). The maximum percentage aggregation was determined during the 10-minute interval after the addition of collagen.

In experiment 7, effects of α-adrenoceptor antagonists on platelet aggregation induced by ADP were examined. An aliquot of PRP was placed in the aggregometer; 1 minute later, 3 antagonists (phentolamine, atipamezole, and yohimbine) at final concentrations of 1 nmol/L to 100 μmol/L were added. Then, 1 minute later, ADP (10 μmol/L) was added (time 0). The maximum percentage aggregation was determined during the 10-minute interval after the addition of ADP.

Examples of representative recorder tracings of feline platelet aggregation in 7 experiments are shown in Figure 2.

Statistical analysis

The statistical analysis was performed with commercially available software (Prism, version 7.0, GraphPad Software Inc, San Diego, Calif). Data were reported as the mean ± SE.

To determine the inhibitory effect of the agents on platelet aggregation, the concentration of an agent at which the response is inhibited by half (IC_{50}) was obtained from the concentration–response curve. The $IC_{50,}$ Concentration of an agent that caused a half-maximal response (ED_{50}), and percentage aggregation data were assessed for normality of distribution with the Shapiro-Wilk test. When the data were normally distributed, the Student *t* test was used for comparisons between agents. When the data were not normally distributed, the Wilcoxon–Mann–Whitney test was used to determine significant differences. The paired *t* test was used to determine significant differences for change in percentage aggregation that were expressed as a percentage of the value for the control agent, which was assigned a value of 100%. For all tests, differences were considered significant at values of $P < 0.05$.

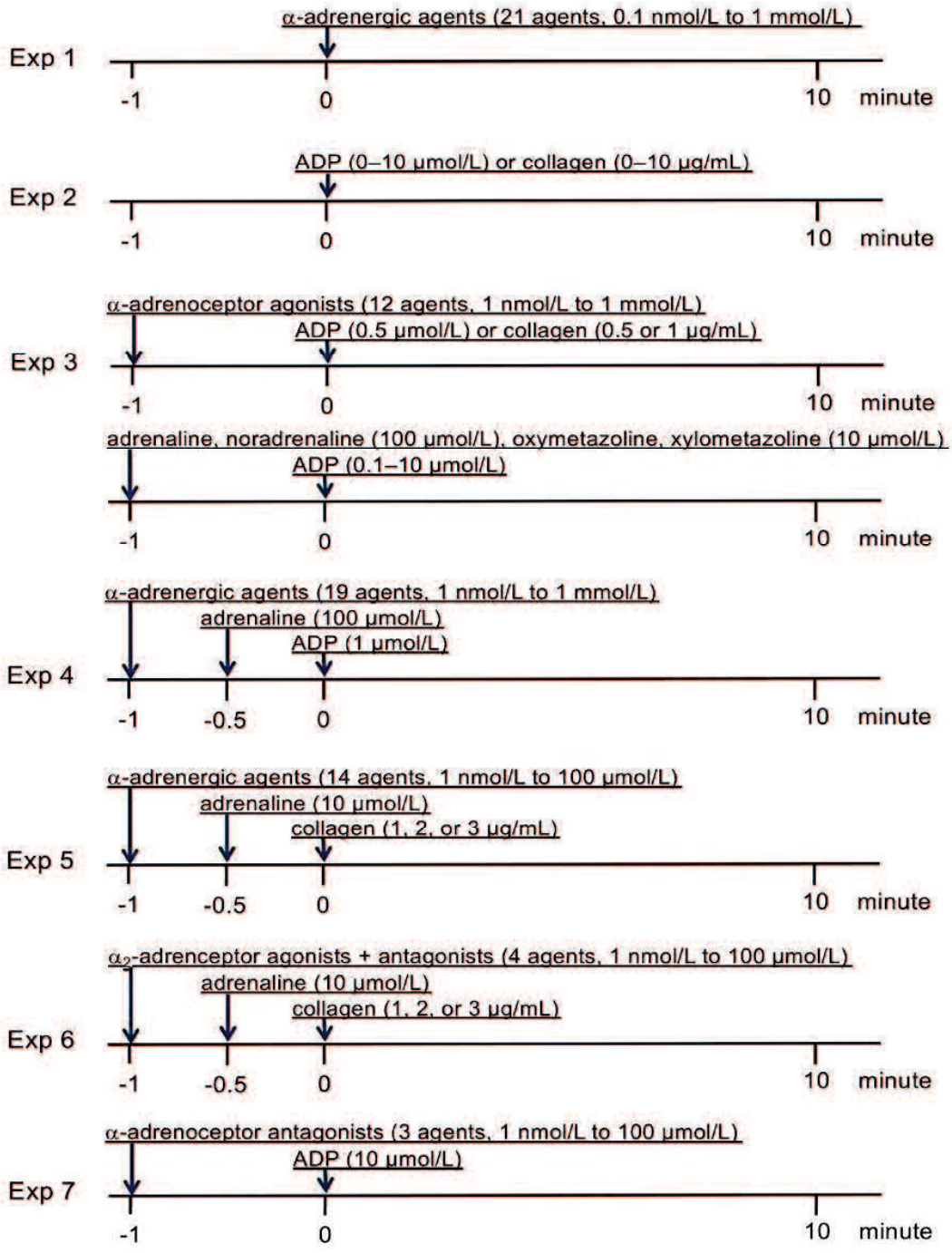

Figure 1. Graphical flow diagram for the protocol of 7 aggregation experiments performed in this study. Exp = experiment.

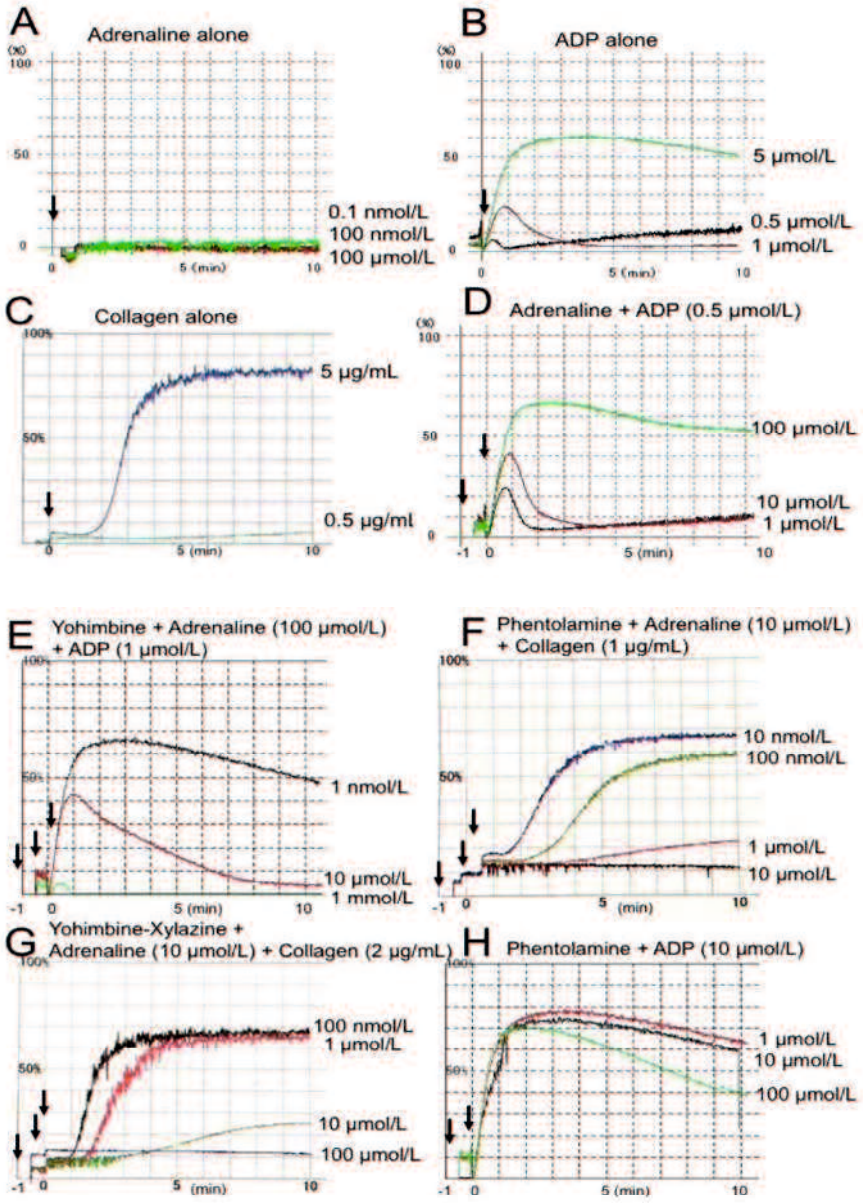

Figure 2. An example of representative recorder tracings of feline platelet aggregation induced by adrenaline alone (A; experiment 1; 0.1 nmol/L, 100 nmol/L, and 100 µmol/L adrenaline), ADP alone (B; experiment 2; 0.5, 1.0 and 5.0 µmol/L ADP), collagen alone (C; experiment 2; 0.5 and 5.0 µg/mL collagen), adrenaline + ADP (D; experiment 3; 1, 10, and 100 µmol/L adrenaline, and 0.5 µmol/L ADP), yohimbine + adrenaline + ADP (E; experiment 4; 1 nmol/L, 10 µmol/L, and 1 mmol/L yobimbine, 100 µmol/L adrenaline, and 1 µmol/L ADP), phentolamine + adrenaline + collagen (F; experiment 5; 10 and 100 nmol/L, and 1 and 10 µmol/L phentolamine, 10 µmol/L adrenaline, and 1 µg/mL collagen), yohimbine-xylazine + adrenaline + collagen (G; experiment 6; 100 nmol/L, 1, 10, and 100 µmol/L yohimbine and xylazine, 10 µmol/L adrenaline, and 2 µg/mL collagen), and phentolamine + ADP (H; experiment 7; 1, 10, and 100 µmol/L phentolamine, and 10 µmol/L ADP).

Results

Effects of α-adrenergic agents on aggregation of feline platelets (experiment 1)

None of the 21 agents tested at concentrations ranging from 0.1 nmol/L to 1 mmol/L elicited aggregation in feline platelets (data not shown).

Effects of ADP or collagen on aggregation of feline platelets (experiment 2)

Both ADP and collagen induced aggregation of feline platelets in a dose-dependent manner (Figure 3). The aggregatory effects of ADP at concentrations exceeding 0.5 μmol/L or of collagen at concentrations exceeding 0.5 μg/mL were significantly different from those of the control agent (saline solution). On the basis of these results, the concentration close to the submaximal amount of aggregation (< 25%) of ADP (0.5 μmol/L) or collagen (0.5 to 1 μg/mL) was chosen to examine the stimulatory effects of α-adrenergic agents on ADP- or collagen-induced platelet aggregation. By contrast, an ADP concentration (10 μmol/L) that induced almost complete platelet aggregation (> 70% aggregation) was chosen to examine the inhibitory effects of α-adrenergic agents on ADP-induced platelet aggregation.

Effects of α-adrenoceptor agonists on aggregation of feline platelets stimulated by ADP or collagen (experiment 3)

Adrenaline and noradrenaline at concentrations of 1 μmol/L to 1 mmol/L potentiated in a dose-dependent manner the platelet aggregation stimulated by a low dose (0.5 μmol/L) of ADP (Figure 4). The maximum and full aggregatory effect for the mean percentage aggregation was observed with adrenaline and noradrenaline at a concentration of 1 mmol/L. However, there was no significant difference in mean aggregation values between adrenaline concentrations of 100

μmol/L and 1 mmol/L and between noradrenaline concentrations of 100 μmol/L and 1 mmol/L. The mean percentage aggregation for adrenaline concentrations of 100 μmol and 1 mmol/L was significantly greater than for adrenaline at a concentration of 10 μmol/L. A small but significantly different potentiation of the ADP-stimulated platelet aggregation was also observed in response to oxymetazoline and xylometazoline at concentrations of 1 to 100 μmol/L, and the maximum aggregatory effect was observed at an oxymetazoline concentration of 1 μmol/L and a xylometazoline concentration of 10 μmol/L. There were no significant differences between oxymetazoline and adrenaline and between xylometazoline and adrenaline at concentrations of 1 or 10 μmol/L. Oxymetazoline and xylometazoline at higher concentrations (100 μmol/L to 1 mmol/L) had an inhibitory effect.

Incubation of platelets with adrenaline (100 μmol/L), noradrenaline (100 μmol/L), oxymetazoline (10 μmol/L), or xylometazoline (10 μmol/L) before the addition of ADP resulted in dose-dependent leftward shifts of the concentration-effect curve for ADP, compared with result for incubation with saline solution before the addition of ADP (Figure 4). The mean ± SE ED_{50} of ADP that caused 50% aggregation was 0.66 ± 0.16 μmol/L after incubation with adrenaline, 0.94 ± 0.17 μmol/L after incubation with noradrenaline, 0.55 ± 0.1 μmol/L after incubation with in oxymetazoline, 0.43 ± 0.1 μmol/L after incubation with xylometazoline, and 1.89 ± 0.18 μmol/L after incubation with the control agent. The ED_{50} values of ADP for these 4 agents were significantly lower than for the control agent. Conversely, clonidine, medetomidine, detomidine, xylazine, naphazoline, tramazoline, phenylephrine, and methoxamine did not potentiate the ADP-stimulated platelet aggregation at concentrations of 1 nmol/L to 1 mmol/L.

Adrenaline potentiated in a dose-dependent manner the platelet aggregation stimulated by collagen (0.5 to 1 mg/mL; Figure 4). The maximum and full aggregatory effect was observed with adrenaline at a concentration of 100 μmol/L.

Effects of imidazoline and nonimidazoline α-adrenergic agents on aggregation of feline platelets induced by the combination of adrenaline and ADP (experiment 4)

The α_2-adrenoceptor antagonist yohimbine and 4 imidazoline α_2-adrenoceptor antagonists (phentolamine, atipamezole, idazoxan, and tolazoline) at concentrations of 1 μmol/L to 1 mmol/L inhibited in a dose-dependent manner the full platelet aggregation induced by the combination of adrenaline at a concentration of 100 μmol/L and ADP at a concentration of 1 μmol/L (Figure 5). By contrast, the nonimidazoline α_1-adrenoceptor antagonist phenoxybenzamine significantly inhibited platelet aggregation induced by adrenaline and ADP only at a high phenoxybenzamine concentration of 100 μmol/L, but the inhibition was less (by approx 65%). Another nonimidazoline α-adrenoceptor antagonist (prazosin) at concentrations up to 100 μmol/L was not effective at inhibiting the adrenaline-ADP induced aggregation. Conversely, 8 imidazoline α-adrenoceptor agonists (oxymetazoline, naphazoline, tramazoline, clonidine, *p*-aminoclonidine, xylometazoline, medetomidine, and detomidine) at concentrations of 1 μmol/L to 1 mmol/L also inhibited in a dose-dependent manner the adrenaline-ADP induced platelet aggregation. Antazoline, an imidazoline devoid of α_2-adrenergic activity, at concentrations of 1 μmol/L to 1 mmol/L also inhibited in a dose-dependent manner the adrenaline-ADP induced platelet aggregation. By contrast, 2 nonimidazoline α-adrenoceptor agonists (xylazine and phenylephrine) significantly inhibited the adrenaline-ADP induced

aggregation at a high concentration of xylazine or phenylephrine of 1 mmol/L, but the inhibition was less (by approx 40%). Another nonimidazoline α-adrenoceptor agonist (methoxamine) at all concentrations was not effective at inhibiting the adrenaline-ADP induced aggregation.

The IC_{50} values obtained for the inhibition of adrenaline-ADP–induced platelet aggregation were summarized **(**Table 1**)**. The order of potencies determined on the basis of the IC_{50} values was as follows: phentolamine > atipamezole > idazoxan > naphazoline > antazoline > xylometazoline, yohimbine, tramazoline, oxymetazoline, and clonidine > medetomidine > tolazoline > detomidine > *p*-aminoclonicine >> phenoxybenzamine. The potencies of atipamezole, idazoxan, and naphazoline for inhibiting aggregation were not significantly different from that of phentolamine. The potencies of yohimbine, oxymetazoline, clonidine, and medetomidine were significantly less (9- to 17-fold difference) from that of phentolamine. The potency of detomidine, *p*-aminoclonidine, and phenoxybenzamine were significantly less (36- to 91-fold difference) from that of phentolamine. The IC_{50} value was not obtained for xylazine, phenylephrine, methoxamine, and prazosin.

Effects of imidazoline and nonimidazoline α-adrenergic agents on aggregation of feline platelets induced by the combination of adrenaline and collagen (experiment 5)

The α_2-adrenoceptor antagonist yohimbine and 4 imidazoline α_2-adrenoceptor antagonists (phentolamine, idazoxan, atipamezole, and tolazoline) at concentrations of 0.1 or 10 μmol/L to 100 μmol/L inhibited in a dose-dependent manner the full platelet aggregation induced by the combination of adrenaline at a concentration of 10 μmol/L and collagen at a concentration of 1 to 3 μg/mL (Figure 6). By contrast, the nonimidazoline α_1-adrenoceptor antagonist prazosin at all concentrations was not effective at inhibiting platelet aggregation induced by adrenaline and

collagen. Conversely, 5 imidazoline α-adrenoceptor agonists (oxymetazoline, naphazoline, clonidine, medetomidine, and dexmedetomidine) at concentrations of 1 μmol/L to 100 μmol/L also inhibited in a dose-dependent manner the adrenaline-collagen–induced aggregation. Antazoline, an imidazoline devoid of α_2-adrenergic activity, at concentrations of 1 μmol/L to 100 μmol/L also inhibited in a dose-dependent manner the adrenaline-collagen–induced platelet aggregation. By contrast, the nonimidazoline α-adrenoceptor agonist xylazine was not effective at inhibiting the platelet aggregation induced by adrenaline and collagen. Similarly, the imidazoline α_2-adrenoceptor agonist moxonidine was not effective at inhibiting the adrenaline-collagen–induced platelet aggregation.

The IC_{50} values obtained for the inhibition of adrenaline-collagen–induced platelet aggregation were summarized (Table 2). The order of potencies determined on the basis of the IC_{50} values was as follows: phentolamine > idazoxan, oxymetazoline, and yohimbine >> naphazoline and clonidine > atipamezole and tolazoline > antazoline > medetomidine and dexmedetomidine. The potencies of idazoxan and oxymetazoline at inhibiting aggregation were not significantly different from that of phentolamine. The potencies of yohimbine, naphazoline, and clonidine were significantly less (5-, 40- and 49-fold difference, respectively) than that of phentolamine. The potencies of atipamezole, tolazoline, and antazoline were significantly less (126- to 458-fold difference) than that of phentolamine. The potencies of medetomidine and dexmedetomidine were significantly less (1,286- and 1,300-fold difference) than that of phentolamine. The IC_{50} value was not obtained for xylazine, moxonidine, and prazosin.

Effects of the combination of α_2-adrenoceptor agonists and antagonists on aggregation of feline platelets induced by adrenaline and collagen (experiment 6)

Concentration-effect curves of α_2-adrenoceptor antagonists and agonists alone and in combination on adrenaline-collagen–induced aggregation of feline platelets were plotted (Figure 7). Mean ± SE IC_{50} values for yohimbine, atipamezole, medetomidine, yohimbine plus xylazine, atipamezole plus xylazine, yohimbine plus medetomidine, and atipamezole plus medetomidine were $3.11 \pm 0.24 \times 10^{-6}$ mol/L, $73.4 \pm 47.7 \times 10^{-6}$ mol/L, $420 \pm 409 \times 10^{-6}$ mol/L, $6.41 \pm 2.82 \times 10^{-6}$ mol/L, $71.7 \pm 61.3 \times 10^{-6}$ mol/L, $3.60 \pm 0.25 \times 10^{-6}$ mol/L, and $257 \pm 160 \times 10^{-6}$ mol/L, respectively; the IC_{50} value was not obtained for xylazine. There were no significant differences in IC_{50} values among yohimbine, yohimbine plus xylazine, or yohimbine plus medetomidine or among atipamezole, atipamezole plus xylazine, and atipamezole plus medetomidine. Therefore, the α_2-adrenoceptor agonists xylazine and medetomidine did not reverse the inhibitory effects of the α_2-adrenoceptor antagonists yohimbine and atipamezole for adrenaline-collagen–induced platelet aggregation.

Effect of phentolamine, atipamezole, and yohimbine on full aggregation of feline platelets induced by ADP alone (experiment 7)

Phentolamine, atipamezole, and yohimbine, which effectively inhibited the full platelet aggregation induced by adrenaline-ADP, were not effective or were less effective at inhibiting the full platelet aggregation induced by ADP (10 μmol/L) alone **(**Figure 8**)**. The IC_{50} value was not obtained for these agents.

Table 1. Mean ± SE potency of imidazoline and nonimidazoline α-adrenergic agents for the inhibition of aggregation of feline platelets induced by adrenaline (100 μM) and ADP (1 μmol/L).

Drug	IC_{50} ($\times 10^{-6}$ mol/L)	IC_{50} ratio*
Phentolamine	2.1 ± 0.4	1
Atipamezole	4.6 ± 0.7	2.2
Idazoxan	6.8 ± 2.0	3.3
Naphazoline	10.4 ± 2.1	5.0
Antazoline	15.9 ± 2.0	7.6
Xylometazoline	18.5 ± 2.8	8.9
Yohimbine	18.6 ± 4.6	8.9
Tramazoline	18.7 ± 3.3	9.0
Oxymetazoline	22.4 ± 6.1	10.7
Clonidine	26.1 ± 4.2	12.5
Medetomidine	36.4 ± 4.0	17.4
Tolazoline	59.7 ± 17.1	28.6
Detomidine	76.0 ± 12.2	36.3
p-Aminoclonidine	90.4 ± 13.1	43.2
Phenoxybenzamine	189.0 ± 24.8	90.6
Xylazine	ND	ND
Phenylephrine	ND	ND
Methoxamine	ND	ND
Prazosin	ND	ND

Each value represents the results for six animals.

* Ratios are in relation to the value for phentolamine, which was assigned a value of 1.0.

ND = Not determined.

Table 2. Mean ± SE potency of imidazoline and nonimidazoline α-adrenergic agents for the inhibition of aggregation of feline platelets induced by adrenaline (10 µmol/L) and collagen (1 to 3 µg/mL).

Drug	IC_{50} ($\times 10^{-6}$ mol/L)	IC_{50} ratio*
Phentolamine	0.69 ± 0.51	1
Idazoxan	1.86 ± 1.08	2.7
Oxymetazoline	2.14 ± 1.94	3.1
Yohimbine	3.23 ± 0.23	4.7
Naphazoline	27.6 ± 16.7	40.0
Clonidine	33.6 ± 21.6	48.7
Atipamezole	87.2 ± 35.0	126
Tolazoline	87.7 ± 36.2	127
Antazoline	316 ± 243	458
Medetomidine	887 ± 565	1286
Dexmedetomidine	897 ± 570	1300
Prazosin	ND	ND
Xylazine	ND	ND
Moxonidine	ND	ND

Each value represents results for blood obtained from 4 or 5 cats.

See Table 1 for the remainder of the key.

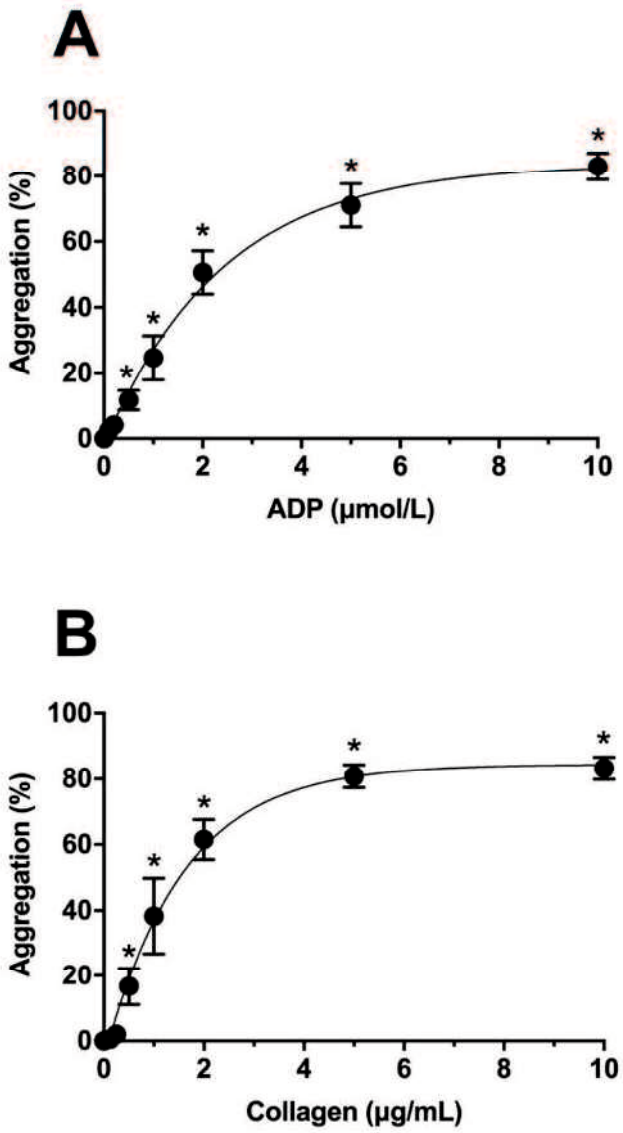

Figure 3. Mean ± SE percentage aggregation of feline platelets induced by ADP (A) and collagen (B). Each value represents results for blood obtained from 6 cats. In each panel, saline (0.9% NaCl) solution was included as a negative control agent. *Value differs significantly ($P < 0.05$) from the value for the control agent.

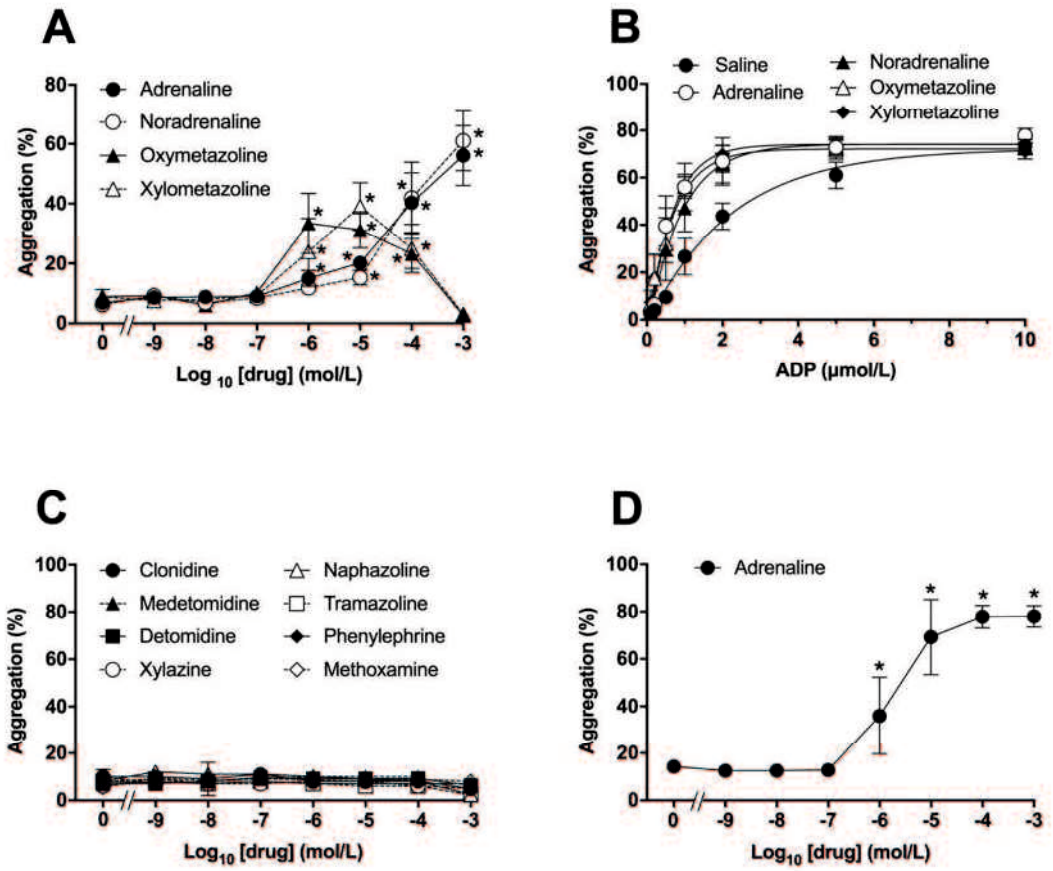

Figure 4. Mean ± SE percentage aggregation of feline platelets. Each value represents results for blood obtained from 6 cats. A—Citrated feline plasma was incubated with various concentrations of adrenaline, noradrenaline, oxymetazoline, xylometazoline, or saline (0.9% NaCl) solution (negative control agent) for 1 minute before the addition of ADP (0.5 µmol/L). B—Citrated feline plasma was incubated with adrenaline (100 µmol/L), noradrenaline (100 µmol/L), oxymetazoline (10 µmol/L), xylometazoline (10 µmol/L), or saline solution for 1 minute before the addition of various concentrations of ADP (0.1 to 10 µmol/L. C—Citrated feline plasma was incubated with various concentrations of α-adrenoceptor agonists for 1 minute before the addition of ADP (0.5 µmol/L). D—Citrated feline plasma was incubated with various concentrations of adrenaline and saline solution for 1 minute before the addition of collagen (0.5 to 1.0 µg/mL). In panels A, C, and D, the value for the negative control solution is 6.2 ± 1.2 to 8.9 ± 1.7 %, 5.5 ± 1.1 to 10.0 ± 3.0 %, and 14.3 ±1.1 %, respectively. Notice that the scale on the y-axis of panel A differs from that of panels B, C, and D. See Figure 3 for remainder of key.

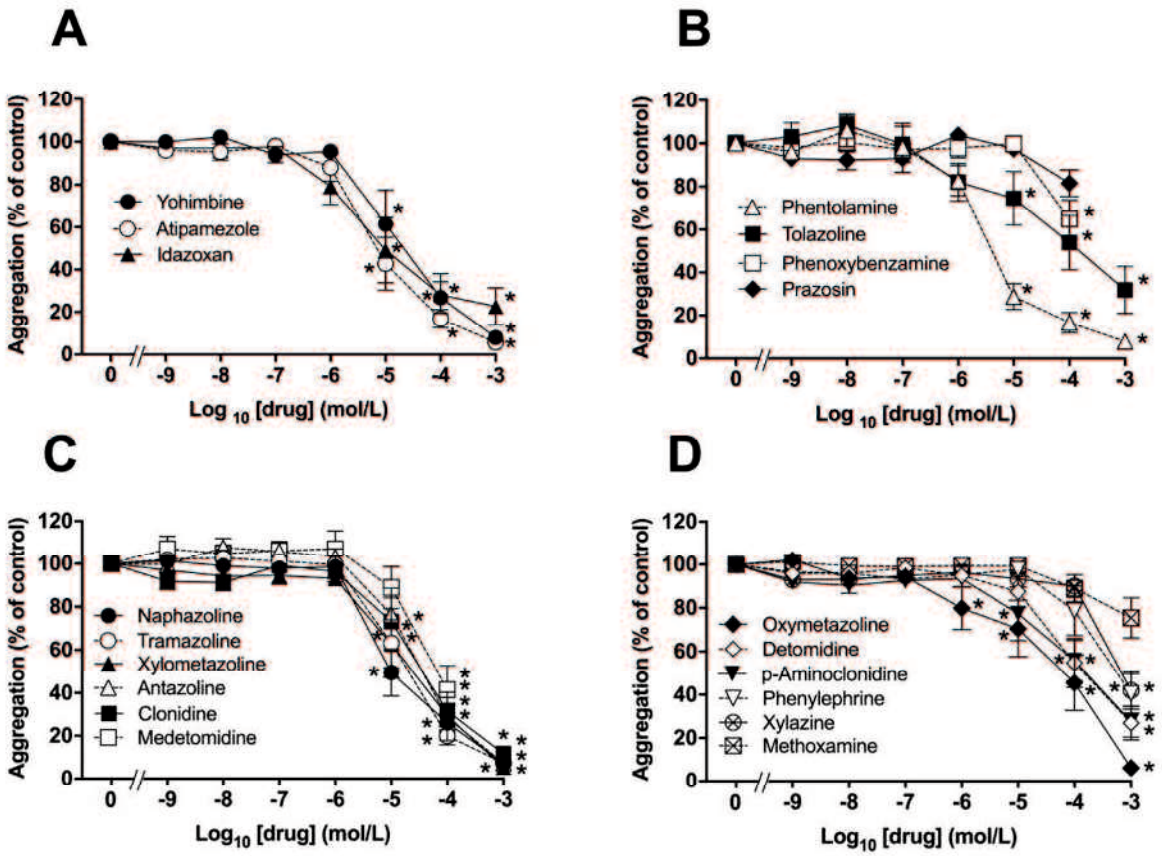

Figure 5. Mean ± SE percentage aggregation for imidazoline and nonimidazoline α-adrenoceptor antagonists (A and B) and agonists (C and D) for feline platelets induced by adrenaline (100 μmol/L) and ADP (1 μmol/L). Each value represents results for blood obtained from 6 cats. Each agent was added to citrated feline plasma; adrenaline was added 0.5 minutes later, and ADP was added 0.5 minutes after that. Values are reported as a percentage of the value for the control agent (percentage aggregation of adrenaline-ADP with saline solution was assigned a value of 100%). See Figure 3 for remainder of key.

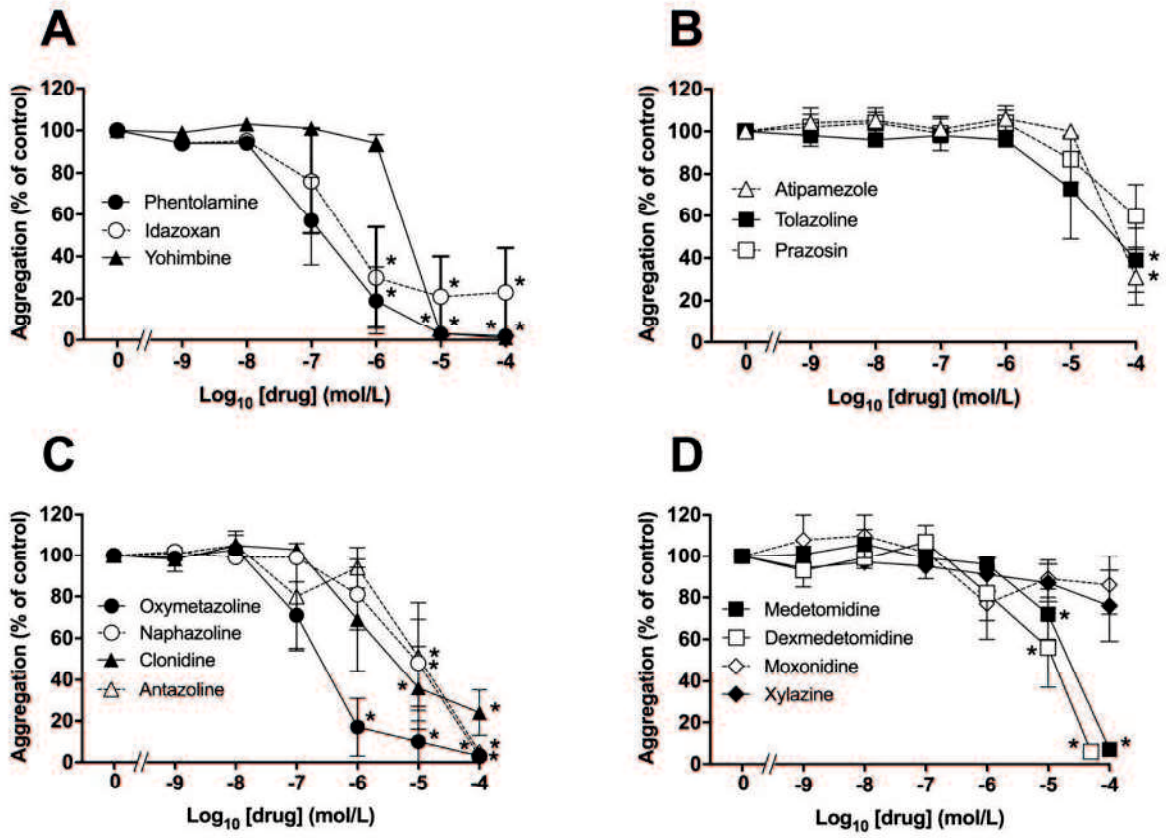

Figure 6. Mean ± SE percentage aggregation for imidazoline and nonimidazoline α-adrenoceptor antagonists (A and B) and agonists (C and D) for feline platelets induced by adrenaline (10 μmol/L) and collagen (1 to 3 μg/mL). Each value represents results of blood obtained from 4 or 5 cats. Each agent was added to citrated feline plasma. adrenaline was added 0.5 minutes later, and collagen was added 0.5 minutes after that. Values are reported as a percentage of the value for the control agent (percentage aggregation of adrenaline-collagen with saline solution was assigned a value of 100%). See Figure 3 for remainder of key.

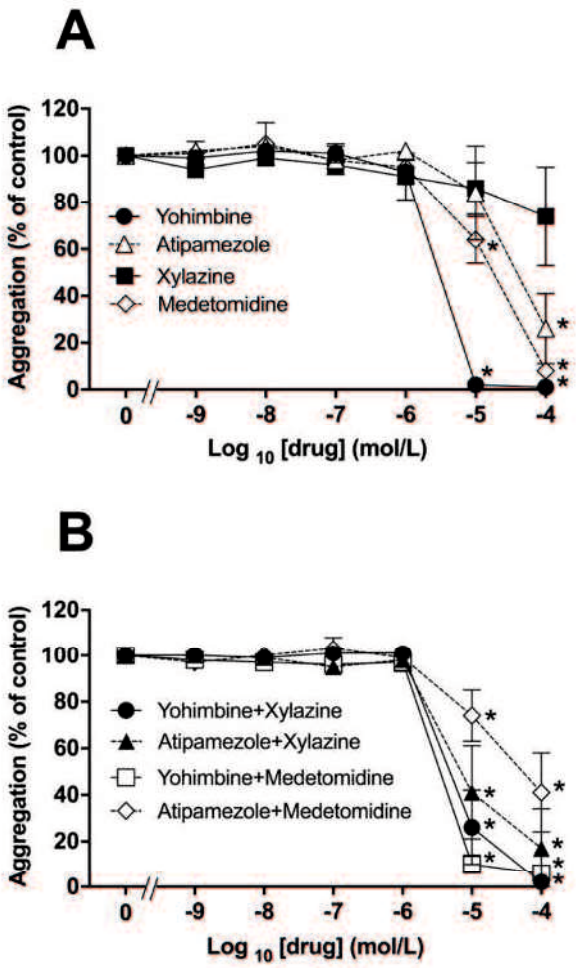

Figure 7. Mean ± SE percentage aggregation for α_2-adrenoceptor agonists and antagonists (A) and the combination of agonists and antagonists (B) for feline platelets induced by adrenaline (10 μmol/L) and collagen (1 to 3 μg/mL). Each value represents results for blood obtained from 4 or 5 cats. Each agent was added to citrated feline plasma. adrenaline was added 0.5 minutes later, and collagen was added 0.5 minutes after that. Values are reported as a percentage of the value for the control agent (percentage aggregation of adrenaline-collagen with saline solution was assigned a value of 100%). See Figure 3 for remainder of key.

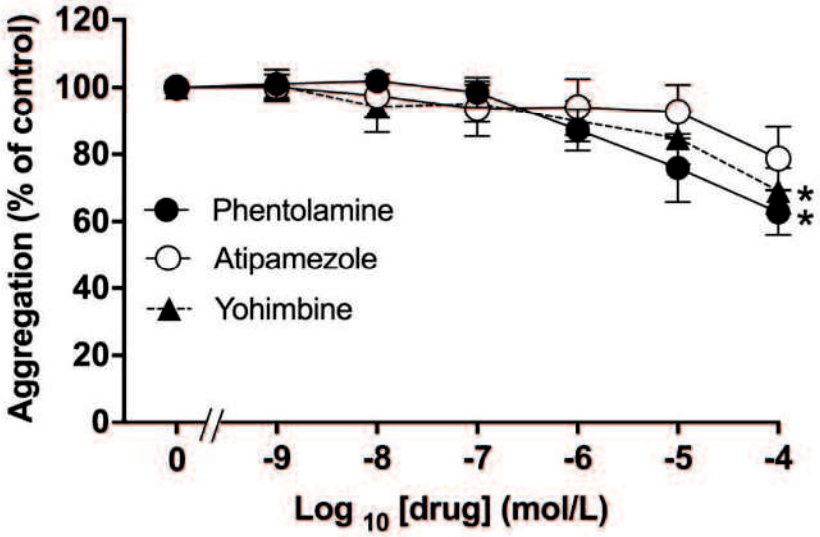

Figure 8. Mean ± SE percentage aggregation for phentolamine, atipamezole, and yohimbine for feline platelets induced by ADP (10 μmol/L) alone. Each value represents results for blood obtained from 6 cats. Each agent was added 1 minute before the addition of ADP. Values are reported as a percentage of the value for the control agent (percentage aggregation of ADP with saline solution was assigned a value of 100%). See Figure 3 for remainder of key.

Discussion

Results of the study reported here confirmed those of previous investigations[22,69] that indicated that adrenaline alone did not induce a change in aggregation of feline platelets and instead potentiated platelet aggregation stimulated by other platelet agonists including ADP and collagen. In addition, results of the present study indicated that noradrenaline potentiated ADP-stimulated platelet aggregation in a dose-dependent manner, and both oxymetazoline and xylometazoline (within limited concentrations, 1 to 100 μmol/L) induced a small potentiation of the ADP-stimulated platelet aggregation in feline platelets. However, other α-adrenoceptor agonists (clonidine, medetomidine, detomidine, xylazine, naphazoline, tramazoline, phenylephrine, and methoxamine) did not induce this potentiating effect. These findings were similar to those for canine platelets.[23] Clonidine reportedly can induce aggregation in human platelets to a limited degree[20,26,48-50,63] and can potentiate ADP-induced platelet aggregation in humans[59] and rabbits.[74] However, the present study found that clonidine did not potentiate ADP-induced aggregation in feline platelets, whereas oxymetazoline and xylometazoline caused a small potentiation of the ADP-induced platelet aggregation, which is in agreement with results with reports of dogs[23] and cattle.[76] The present results, in combination with results of the aforementioned report,[20,23,26,48-50,59,63,75,76] highlight species-specific variations in the potentiation of platelet aggregation.

Platelet α-adrenoceptors in humans have been characterized pharmacologically as Gi-coupled α_2-adrenoceptors of the α_{2A}-subtype, although a decrease in cAMP alone may not be the only cause of aggregation.[8,9,46,57,64,71,74] In domestic animals, binding experiments with radiolabeled adrenoceptor agonists and antagonists have revealed the expression of α_2-

adrenoceptors on canine, feline, leporine, and murine platelets but not on bovine or equine platelets.[24] The density of platelet α_2-adrenoceptors evaluated by the use of specific tritiated yohimbine binding is reportedly lower in cats than in dogs[24] and humans.[8] In the present study, adrenaline-potentiated aggregation of feline platelets stimulated by low concentrations of ADP or collagen was inhibited in a dose-dependent manner by the α_2-adrenoceptor antagonists atipamezole, yohimbine, phentolamine, idazoxan, and tolazoline, whereas the α_1-adrenoceptor antagonists phenoxybenzamine and prazosin were not effective or were less effective at inhibiting the adrenaline-potentiated aggregation. In previous reports,[15,35,47] the order of affinity of antagonists for α_2-adrenoceptors is atipamezole > yohimbine > idazoxan > phentolamine > tolazoline > prazosin. In the study reported here, the order of potency of α_2-adrenoceptor agents for the inhibition of adrenaline-potentiated platelet aggregation was not in agreement with the order of affinity values for α_2-adrenoceptors. However, although the mechanism for adrenaline-induced intraplatelet signaling is unclear, adrenaline is known to increase the release of arachidonic acid from platelet membranes via the phosphorylation of p38 mitogen-activated protein kinase and cytosolic phospholipase A_2 via the α_{2A}-adrenoceptors and its Na^+-effector sites.[46] In addition, adrenaline-potentiated platelet aggregation is not mediated by β-adrenoceptors because the β-adrenoceptor antagonist propranolol is less effective at inhibiting adrenaline-ADP–induced aggregation.[29] Therefore, results of the present study suggested a partial involvement of the α_2-adrenoceptor mediated-cascade in aggregation of feline platelets and the simultaneous involvement of other pathways in addition to the involvement of α_2-adrenoceptors. Furthermore, in the present study, certain imidazoline or α_2-adrenoceptor antagonists (or both) were able to completely inhibit the full platelet aggregation induced by adrenaline-ADP or adrenaline-collagen but were not effective or were less effective at inhibiting

full platelet aggregation induced by ADP alone. These findings suggested that the inhibitory effect of imidazoline agents on feline platelet aggregation is more specific for the action of adrenaline via α_2-adrenoceptors rather than the action of ADP as a inducer of platelet aggregation.

In the present study, imidazoline or α_2-adrenoceptor agonists including naphazoline, antazoline, xylometazoline, tramazoline, oxymetazoline, clonidine, medetomidine, dexmedetomidine, detomidine, and *p*-aminoclonidine, but not moxonidine, caused dose-dependent inhibition of adrenaline-ADP– or adrenaline-collagen–induced aggregation of feline platelets. These results in feline platelets were extremely similar to results in canine platelets, except for a different order for α_2-adrenoceptor activity,[23] and to results in human platelets.[53] The imidazoline agent antazoline, which lacks α_2-adrenoceptor activity, inhibited adrenaline-potentiated platelet aggregation, which suggested that it interacts with non–α_2-adrenoceptor sites on feline platelets. Furthermore, in the present study, neither xylazine nor medetomidine reversed the inhibitory effects of the α_2-adrenoceptor antagonists yohimbine and atipamezole for adrenaline-collagen–induced platelet aggregation, which suggested that both yohimbine and atipamezole also interacted with non–α_2-adrenoceptor sites on feline platelets. On the basis of these results, it would be difficult to envisage how the α_2-adrenoceptors could be the mediator of the observed responses. Both feline platelets and canine platelets have nonadrenergic I_1 receptor sites (as determined by the use of labeled tritiated clonidine) and I_2 receptor sites (as determined by the use of labeled tritiated idazoxan).[24] The order of potency of imidazoline agents for the inhibition of adrenaline-potentiated platelet aggregation in the present study appeared to be in agreement with the order of affinity values for platelet I_1 receptors or I_2 receptors.[49] In a comparative study[77] of the effects of imidazoline α-adrenergic agents on intraplatelet cAMP and

thromboxane B_2 that involved the use of canine platelets with both I_1 and I_2 receptors and leporine platelets that lacked I_1 receptors, it was suggested that imidazoline α_2-adrenergic agents suppress cAMP production via the α_2-adrenoceptor while exerting a negative effect on generation of thromboxane B_2 via the arachidonic acid–thromboxane A_2 pathway. Therefore, it would seem logical to conclude that imidazoline agents inhibit platelet aggregation via nonadrenoceptor binding sites, including I_1 and I_2 receptors, on feline platelets.

Yohimbine and idazoxan exhibit only modest selectivity for rat α_2-receptors, compared with selectivity for 5-Hydroxytryptamine$(5HT)_{1A}$ receptors.[28,73] Oxymetazoline also stimulates 5HT receptors, including $5HT_{1A}$, and can mobilize a second signaling system.[44,58] Furthermore, noradrenaline induces heterologous desensitization of the $5HT_1$ receptors in human platelets through activation of protein kinase C.[68] Therefore, it is also possible that the effect of imidazoline or α-adrenergic agents on platelet aggregation may be partially mediated by serotonin receptors, including $5HT_{1A}$. In the present study, both oxymetazoline and xylometazoline induced a small potentiation of the ADP-induced platelet aggregation, but both agents at higher concentrations had an inhibitory effect on potentiation of the ADP-induced platelet aggregation. Although the precise mechanism for this effect was unknown, it may have been attributable to the complicated actions via 5HT receptors, α_2-adrenoceptors, and I_1 and I_2 receptors on feline platelets.[13,24]

Several drugs with α_2-adrenoceptor activity are clinically available. For felids, the α_2-adrenoceptor agonists xylazine, medetomidine, and dexmedetomidine are used for sedation and analgesia and as a premedication for general anesthesia, whereas the antagonists atipamezole and yohimbine are used to reverse the effects of the aforementioned α_2-adrenoceptor agonists. On the basis of the pharmacokinetic data for xylazine, medetomidine, and dexmedetomidine, which

have typically been administered systemically at clinically recommended doses to cats and dogs,[18,30,54] results for the present study indicated that the α_2-adrenoceptor agonists xylazine, medetomidine, and dexmedetomidine may be used in cats with minimal concern for adverse effects on platelet function and hemostasis because xylazine did not inhibit platelet aggregation and both medetomidine and dexmedetomidine did not inhibit in vitro platelet aggregation at the estimated blood concentrations of both agents in clinical use. However, the α_2-adrenoceptor antagonists phentolamine, yohimbine, and atipamezole may also have inhibitory effects on feline hemostasis during certain events (eg, blood vessel damage and collagen exposure). The study reported here represented results of in vitro experiments. Therefore, it will be necessary to investigate the effects of various agents on aggregation of feline platelets in vivo or ex vivo.

It has been suggested that overactivity of the sympathetic nervous system and increased catecholamine concentrations may have influence hemostasis via actions on platelets, coagulation and fibrinolytic factors, and endogenous anticoagulants, thereby leading to activation of both the coagulation and fibrinolytic systems.[62] Hypercatecholaminemia occurs in conditions such as pheochromocytoma,[72] endotoxin shock,[16,21] and acute stress[56] in cats. Risk factors for poor short-term survival in dogs with pheochromocytoma involve disseminated intravascular coagulation.[6] Fatal thromboembolism has been also reported in a cat with pheochromocytoma.[10] In addition, low-dose endotoxin infusion induces platelet aggregation,[14] and intravascular coagulation is manifested during endotoxin shock in cats.[34] Therefore, the results of the study reported here suggested that imidazoline α-adrenergic agents may have clinical benefits for the hypercoagulatory state that accompanies hypercatecholaminemia or for the conditions in which there is platelet reactivity to adrenaline because catecholamines have a stimulatory effect on platelet aggregation. However, further studies will be required to examine the effects of α-

adrenergic agents on in vivo or ex vivo platelet aggregation under various pathological conditions in cats.

In the present study, both adrenaline and noradrenaline potentiated in a dose-dependent manner aggregation of feline platelets induced by ADP or collagen, but other α-adrenoceptor agonists, except for oxymetazoline and xylometazoline, did not potentiate platelet aggregation induced by ADP. Furthermore, results indicated that the α_2-adrenoceptor antagonists or certain imidazoline α-adrenergic agents (or both) inhibited, in a dose-dependent manner, adrenaline-potentiated aggregation induced by ADP or collagen, whereas α_1-adrenoceptor antagonists and nonimidazoline α-adrenergic agents were ineffective or less effective at inhibiting adrenaline-potentiated aggregation and that the α_2-adrenoceptor agonists medetomidine and xylazine did not reverse the inhibitory effects of the α_2-adrenoceptor antagonists atipamezole and yohimbine on adrenaline-potentiated aggregation. Furthermore, the results suggested that adrenaline-potentiated aggregation was mediated by α_2-adrenoceptors, whereas imidazoline agents inhibited platelet aggregation via imidazoline receptors in cats. Results of the present study also suggested that clinically recommended doses of xylazine, medetomidine, and dexmedetomidine may be used in feline practice with minimal concern for adverse effects on platelet function, although further in vivo or ex vivo studies will be required to examine the effects of these agents on platelet aggregation.

Chapter 2

Effects of systemic administrations of medetomidine and xylazine on *ex vivo* platelet aggregation in clinically normal cats

Introduction

In practice, the α_2-adrenoceptor agonists medetomidine (MED) and xylazine (XYL) are widely used to produce reliable sedation, analgesia, and muscle relaxation in cats.[33] Although both drugs are used similarly in practice, there are differences between them. MED is a more potent, selective, and specific α_2-adrenoceptor agonist compared with XYL. The ratio of α_2-adrenoceptor selectivity to α_1-adrenoceptor selectivity of MED (1,620:1) is approximately 10-fold as great as that of XYL (160:1).[70] In addition, MED, in contrast to XYL, has an imidazole (I) ring that has an affinity for I-receptors.[43]

In cats, overactivity of the sympathetic nervous system and increased catecholamine concentrations are induced in conditions such as pheochromocytoma,[72] endotoxin shock,[17] and acute stress.[56] Hypercatecholaminemia reportedly has an influence on hemostasis, including disseminated intravascular coagulation[6] and thromboembolism[10,14,62] by acting on platelets. Because both MED and XYL reduce plasma concentrations of adrenaline and noradrenaline in cats[27] it may be important on blood homeostasis to examine the platelet response in cats administered systemically with MED or XYL.

Regarding in vitro platelet responses to catecholamines in small animals, adrenaline alone does not induce platelet aggregation; however, it potentiates platelet aggregation stimulated by other platelet agonists including ADP, collagen, and thrombin in dogs,[23] cats[22,36] and rabbits.[75] It has been reported that adrenaline-potentiated platelet aggregation is mediated by α_2-adrenoceptors on platelets, because this is blocked by α_2-adrenoceptor antagonists but not by α_1-adrenoceptor antagonists in dogs,[23] cats[36] and rabbits[75] as well as in humans.[53] However, it has been reported that many I α_2-adrenoceptor agents inhibit adrenaline-potentiated platelet

aggregation in dogs,[23] cats[36] and rabbits.[75] Nonadrenergic I_1 and I_2 receptors that are pharmacologically distinct from α_2-adrenoceptors have been expressed in canine and feline platelets[24] as well as in human platelets.[51,52] In addition, the densities of I_1 and I_2 receptors and α_2-adrenoceptors differed among animal species.[24] MED binds to I_1 and I_2 receptors on feline platelets, whereas XYL does not have an affinity for I_1 and I_2 receptors.[24] A recent in vitro study has reported that medetomidine inhibited adrenaline-potentiated platelet aggregation induced by ADP or collagen in a dose-dependent manner, but XYL was ineffective in inhibiting adrenaline-potentiated aggregation.[36] However, to the best of our knowledge, there are no published reports on the blood platelet aggregation in cats that were administered XYL or MED systemically. Therefore, this study was conducted to compare the effects of MED and XYL administered intramuscularly (IM) on *ex vivo* platelet aggregation in healthy cats.

Materials and methods

Animals

Two healthy male (2 neutered) and 3 healthy female (2 neutered) adult mixed-breed cats with a mean age of 9.2 years (standard deviation [SD] = 3.3) and a mean weight of 3.8 kg (SD = 0.56) were used in this study. They were fed a standard commercial dry food formulated for cats and raised in a laboratory with appropriate animal management facilities. Physical examination and hematologic analysis prior to the experiments revealed that all cats were clinically normal. The animals' signalments, some hematologic and blood biochemical profiles are summarized in Table 3. The study protocol was approved by the Animal Research Committee of Tottori University.

Table 3. Summary of signalments, hematologic and blood biochemical profiles in 5 cats used in this study.

Cat	1	2	3	4	5	Reference range
Breed	Mixed	Mixed	Mixed	Mixed	Mixed	
Gender	Female intact	Female ovariohysterectomized	Female ovariohysterectomized	Male castrated	Male castrated	
Age (year)	12	11	8	11	4	
Body weight (kg)	3.1	4.3	3.4	3.8	4.4	
Clinical signs	Healthy	Healthy	Healthy	Healthy	Healthy	
Packed cell volume (%)	32	34	40	38	42	30-45
Red blood cell counts ($\times 10^4/mm^3$)	623	631	865	754	889	500-1,000
White blood cell counts ($\times 10^2/mm^3$)	123	107	119	116	98	55-195
Platelet counts ($\times 10^4/mm^3$)	45	41	39	33	56	30-80
Plasma protein (mg/dL)	7.4	6.7	6.8	7.3	7.2	5.7-7.8
Blood glucose (mg/dL)	89	109	113	90	94	71-148
Total cholesterol (mg/dL)	166	109	99	121	140	89-176
Aspartate aminotransferase (IU/L)	48	32	36	40	26	18-51
Alanine aminotransferase (IU/L)	79	61	60	57	41	22-84
Blood urea nitrogen (mg/dL)	30	26	25	30	21	17.6-32.8
Creatinine (mg/dL)	1.5	1.4	1.1	1.3	1.3	0.8-1.8

Experimental procedures

Five cats were used repeatedly for 7 treatment groups (5 cats per each group) in a modified randomized design. In group 1, each cat was administered a physiological saline solution (0.1 mL/kg) IM as the nonmedicated control. In group 2, 3, 4, 5, 6, and 7, each cat received IM 0.5, 2.0, and 4.0 mg/kg XYL hydrochloride (Sigma-Aldrich Japan K.K., Tokyo, Japan), and 20, 80, and 160 μg/kg MED hydrochloride (Dorbene, Syva Laboratorios, S.A., Spain), respectively. Seven groups were denoted as control, XYL-0.5, XYL-2, XYL-4, MED-20, MED-80, and MED-160. Cat-1 was treated with control, XYL-0.5, MED-20, XYL-2, MED-80, XYL-4, and MED-160 in that order. Cat-2 was treated in the order of XYL-0.5, MED-20, XYL-2, MED-80, XYL-

4, MED-160, and control. Cat-3 was treated in the order of MED-20, XYL-2, MED-80, XYL-4, MED-160, control, and XYL-0.5. Cat-4 was treated in the order of XYL-2, MED-80, XYL-4, MED-160, control, XYL-0.5, and MED-20. Cat-5 was treated in the order of MED-80, XYL-4, MED-160, control, XYL-0.5, MED-20, and XYL-2.

Intervals between treatments ranged from 1 to 4 weeks for each cat in this study. The intervals were 1 to 2 weeks after control, lowest and middle-dose MED or XYL treatments, and 2 to 4 weeks after highest-dose MED or XYL treatments. The washout period (mean ± SD, week) between treatments was 2.3 ± 1.0 in Cat-1, 2.5 ± 0.8 in Cat-2, 2.3 ± 1.0 in Cat-3, 2.5 ± 1.2 in Cat-4, 2.3 ± 0.5 in Cat-5, respectively. The interval of 1 week between treatments was set three times only, once in 3 cats in this study. In each case, the 1-week interval was only between the control and XYL-0.5 treatments.

Food and water were withheld for 12 h before the start of each experiment. Food and water were provided after sample collection at 8 h after injection. The experiments were done in a room where the room temperature was maintained at 25°C.

Blood sampling and preparation of citrated platelet plasma

Jugular blood samples (4.5 mL) were collected in plastic syringes containing 3.2% sodium citrate solution at a ratio of 1 part anticoagulant to 9 parts blood, 4 times (immediately before injection of the treatment [0 h; baseline] and 2, 4, and 7 h after injection) from each cat. The citrated blood was centrifuged at 90 to 110 × *g* for 10 to 15 min to obtain platelet-rich plasma (PRP). The platelet-poor plasma (PPP) was obtained by centrifuging the remaining citrated blood after collecting PRP at 1,500 × *g* for 15 min. The final platelet count in PRP was adjusted to 25 to 30 × 10^4 platelets/μL via dilution with autologous PPP.

The determination of time points of measurement is involved the technical aspects of measuring platelet aggregation, since the aggregation test must be performed immediately after blood sampling. It takes approximately 1 to 1.5 h to complete the preparation of citrated platelet plasma and the platelet aggregation experiment after one blood sampling. So, we chose an interval of at least 2 h before the next blood sampling. In addition, after administration of the highest doses of MED and XYL, the cat was deeply sedated at 2 h, and sedation was continued for 4 h, and disappeared at 7 h. For these reasons, we decided 4 time points of the measurement at 0, 2, 4, and 7 h after injection.

Aggregation experiments

The platelet aggregation experiments were performed as previously described.[23,75] Briefly, a turbidimetric method was used. The percent aggregation was determined after adding the aggregation agent and was standardized via the assumption that PPP and PRP represented 100% and 0% light transmission, respectively. In each PRP sample, the aggregation effects of ADP and collagen were examined as follows. An aliquot (200 μL) of PRP was placed in an aggregometer (MCM Hema Tracer 804, LMS Co Ltd, Tokyo, Japan) at 37ºC, and 1 min later, an aliquot (22 μL) of ADP (0, 1, 3, and 5 μmol/L) or collagen (0, 1, 3, and 5 μg/mL) was added to the PRP, and the maximum percentage aggregation was recorded during the subsequent 10-min interval.

Statistical analysis

Statistical analysis was performed using commercially available statistical programs (Prism 7.0, GraphPad Software Inc, San Diego, CA). Data were reported as the mean ± standard error (SE). To determine the potency of the platelet aggregatory effect of ADP or collagen, the mean

effective dose (ED) 50 that caused 50% aggregation was obtained from the concentration-response curve on platelet aggregation. The ED50 and percent aggregation data were assessed for normality of distribution with the Shapiro-Wilk test. When the data were normally distributed, the paired *t* test was used for comparisons between the groups at 0, 2, 4, and 7 h after injection of XYL or MED. When the data were not normally distributed, the Wilcoxon-Mann-Whitney test was used to determine significant differences. The paired *t* test was used to determine significant differences for change in percentage aggregation that were expressed as a percentage of the value for the time 0 h (baseline), which was assigned a value of 100%. For all tests, differences were considered significant at values of $P < 0.05$.

Results

ADP-induced platelet aggregation response

In the control, XYL-0.5, XYL-2, XYL-4, MED-20, MED-80, and MED-160 groups, 3 μmol/L ADP-induced maximum platelet aggregations were 76.2 ± 6.2%, 78.0 ± 3.4%, 80.2 ± 5.1%, 79.6 ± 0.9%, 78.0 ± 3.5%, 81.8 ± 2.4%, and 79.4 ± 3.1% before XYL or MED injection (0 h; baseline), respectively. There were no significant differences in the maximum aggregation between the groups at the baseline. In the control and all of the XYL groups, no significant differences for change in percentage aggregation, which was expressed as a percentage of the value for the baseline, were observed at 2, 4, and 7 h after saline or XYL injection (Figure 9A). In addition, there were no significant differences in percentage aggregation between XYL and control groups at 2, 4, and 7 h. In contrast, a significant decrease in the percentage aggregation was observed at 2, 4, and 7 h compared with the baseline in the MED-80 group, but not in the

MED-20 and MED-160 groups (Figure 9B). The percentage aggregation was significantly lower in the MED-80 group than in control group at 2, 4, and 7 h. There were no significant differences in percentage aggregation between MED-20 and control groups and between MED-160 and control groups at any time.

The maximum platelet aggregation induced by 5 μmol/L ADP was 84.0 ± 3.1%, 76.8 ± 3.8%, 86.8 ± 2.0%, 81.2 ± 1.0%, 79.2 ± 2.6%, 83.2 ± 2.0%, and 82.6 ± 3.8% at the baseline in the control, XYL-0.5, XYL-2, XYL-4, MED-20, MED-80 and MED-160 groups, respectively. There were no significant differences in the maximum aggregation between the groups at the baseline. Aggregation responses induced by 5 μmol/L ADP after XYL or MED injection were similar to those by 3 μmol/L ADP. In the control and all of the XYL groups, no significant changes in percentage aggregation were observed at any time after saline or XYL injection (Figure 10A). In the MED-80 group, the aggregation induced by 5 μmol/L ADP decreased at 2, 4, and 7 h compared with the baseline after MED injection (Figure 10B). The percentage aggregation was significantly lower in the MED-80 group than in the control group at 2, 4, and 7 h. In the MED-20 and MED-160 groups, the aggregation induced by 5 μmol/L ADP did not significantly decrease at 2–7 h compared with the baseline after MED injection (Figure 10B).

The ED50 values of ADP that caused 50% platelet aggregation in all groups are summarized in Table 4. The ED50 of ADP in the MED-80 group was significantly higher at 2–7 h than at the baseline. In addition, the ED50 value of ADP in the MED-80 group at 2 h was significantly higher than that of the control, XYL-2, and XYL-4 groups. There were no significant differences in ED50 values between the other groups, although the ED50 value in MED-20 and MED-160 tended to be higher than that of the control at 2 h and 7 h, respectively.

Collagen-induced platelet aggregation response

In the control, XYL-0.5, XYL-2, XYL-4, MED-20, MED-80, and MED-160 groups, 3 μg/mL collagen-induced maximum platelet aggregation at the baseline was 77.6 ± 6.4%, 80.8 ± 3.8%, 81.8 ± 5.9%, 84.0 ± 2.0%, 81.6 ± 4.7%, 83.4 ± 2.9%, and 84.0 ± 3.2%, respectively. There were no significant differences in the aggregations between the groups at the baseline. In the control and all of the XYL groups, 3 μg/mL collagen-induced aggregation did not significantly change at 2, 4, and 7 h after saline or XYL injection compared with the baseline (Figure 11A). In contrast, a significant decrease in the percentage aggregation was observed at 2 and 4 h compared with the baseline in the MED-80 group, but not in the MED-20 and MED-160 groups (Figure 11B). The percentage aggregation was significantly lower in MED-80 than in control at 2 h, but there were no significant differences between XYL and control groups or and between MED-20 or MED-160 and control groups at any time.

In the control, XYL-0.5, XYL-2, XYL-4, MED-20, MED-80, and MED-160 groups, 5 μg/mL collagen-induced maximum platelet aggregation at the baseline was 79.2 ± 7.7%, 81.2 ± 3.9%, 83.4 ± 5.7%, 84.2 ± 2.3%, 80.8 ± 4.1%, 84.8 ± 3.3%, and 84.0 ± 4.4%, respectively. There were no significant differences in the aggregations between the groups at the baseline. In the control and all of the XYL groups, 5 μg/mL collagen-induced aggregation did not significantly change at 2, 4, and 7 h after saline or XYL injection compared with the baseline (Figure 12A). In contrast, a significant decrease in the percentage aggregation was observed at 2 and 4 h compared with the baseline in the MED-80 group but not in the MED-20 and MED-160 groups (Figure 12B). The percentage aggregation was significantly lower in MED-80 than control at 2 h, but there were no significant differences between XYL and control groups and between MED-20 or MED-160 and control groups at any time.

The ED50 values of collagen that caused 50% platelet aggregation in all groups are summarized in Table 5. The ED50 of collagen in the MED-80 group was significantly higher at 2 and 4 h than at the baseline. In addition, the ED50 value of collagen in the MED-80 group at 2 h was significantly higher than that of the control and the MED-160 groups. There were no significant differences in ED50 value between the other groups, although ED50 value in XYL-4 tended to be higher than that of the control at 2 h.

Table 4. Mean ± standard error (SE) mean effective dose (ED) 50 (ED50) of adenosine diphosphate (ADP; μmol/L) that caused 50% aggregation on *ex vivo* platelet aggregation in 5 cats administered xylazine or medetomidine intramuscularly.

Group	Time (h)			
	0	2	4	7
Control	1.65 ± 0.25	1.63 ± 0.20	1.63 ± 0.21	1.43 ± 0.17
XYL-0.5	1.87 ± 0.20	1.66 ± 0.21	1.72 ± 0.15	1.70 ± 0.14
XYL-2	1.81 ± 0.29	1.47 ± 0.28	1.68 ± 0.16	1.50 ± 0.22
XYL-4	1.26 ± 0.33	1.49 ± 0.23	1.42 ± 0.29	1.60 ± 0.22
MED-20	1.92 ± 0.17	2.24 ± 0.34	1.52 ± 0.22	1.81 ± 0.12
MED-80	1.70 ± 0.13	2.45 ± 0.25*†§‡	2.07 ± 0.18*	2.22 ± 0.15*
MED-160	1.42 ± 0.30	1.52 ± 0.15	1.77 ± 0.17	1.86 ± 0.11

* $P < 0.05$, significantly different from 0 h (baseline).

† $P < 0.05$, significantly different from control.

§ $P < 0.05$, significantly different from XYL-2.

‡ $P < 0.05$, significantly different from XYL-4.

Table 5. Mean ± standard error (SE) mean effective dose (ED) 50 (ED50) of collagen (μg/mL) that caused 50% aggregation on ex vivo platelet aggregation in 5 cats administered xylazine or medetomidine intramuscularly.

Group	Time (h)			
	0	2	4	7
Control	1.61 ± 0.37	1.86 ± 0.11	2.20 ± 0.38	2.00 ± 0.30
XYL-0.5	1.69 ± 0.38	2.03 ± 0.07	1.90 ± 0.11	1.88 ± 0.10
XYL-2	1.99 ± 0.35	1.89 ± 0.18	1.84 ± 0.16	1.53 ± 0.32
XYL-4	1.53 ± 0.28	2.13 ± 0.25	2.09 ± 0.22	1.66 ± 0.27
MED-20	1.42 ± 0.40	1.72 ± 0.60	1.47 ± 0.44	1.43 ± 0.39
MED-80	1.83 ± 0.11	2.48 ± 0.23*†§	2.27 ± 0.21*	2.14 ± 0.28
MED-160	1.50 ± 0.19	1.60 ± 0.16	1.54 ± 0.33	1.89 ± 0.17

* $P < 0.05$, significantly different from 0 h (baseline).

† $P < 0.05$, significantly different from control.

§ $P < 0.05$, significantly different from MED-160.

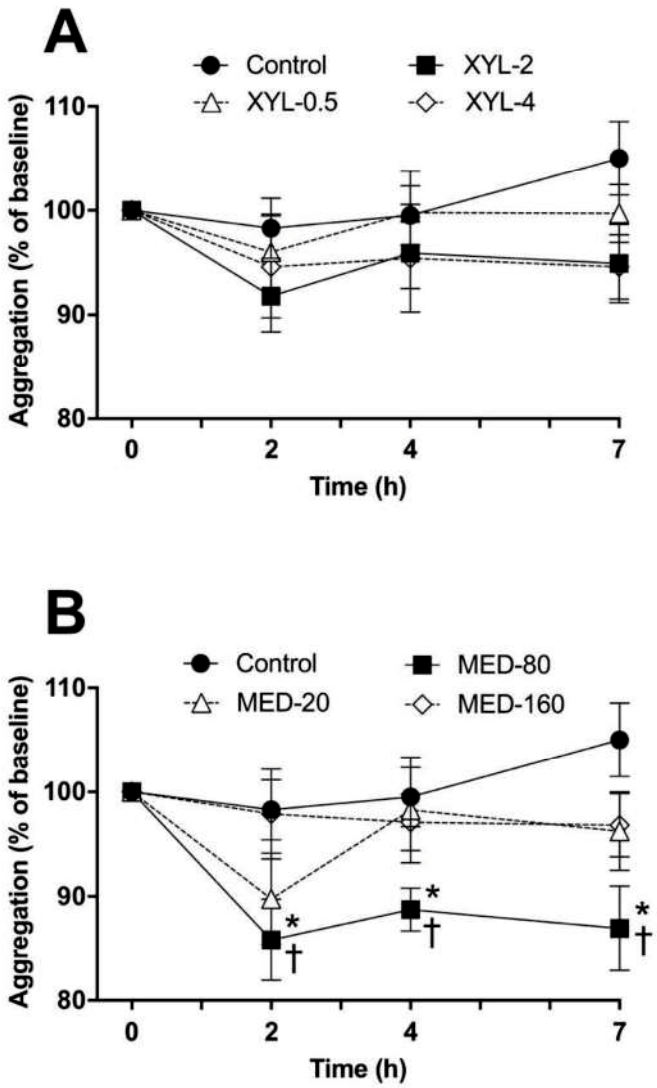

Figure 9. Mean ± standard error (SE) percentage changes of ex vivo platelet aggregation induced by 3.0 μmol/L adenosine diphosphate (ADP) after an intramuscular administration of xylazine (A) and medetomidine (B) in 5 cats. Values are reported as a percentage of the value for the 0 h (percentage aggregation of ADP at 0 h was assigned a value of 100%). Control, XYL-0.5, XYL-2, XYL-4, MED-20, MED-80, and MED-160 groups showed physiological saline solution, 0.5, 2.0, 4.0 mg/kg xylazine, 20, 80, and 160 μg/kg medetomidine, respectively. *Value differs significantly ($P < 0.05$) from the value for the 0 h. †Value differs significantly ($P < 0.05$) from the value for the control group.

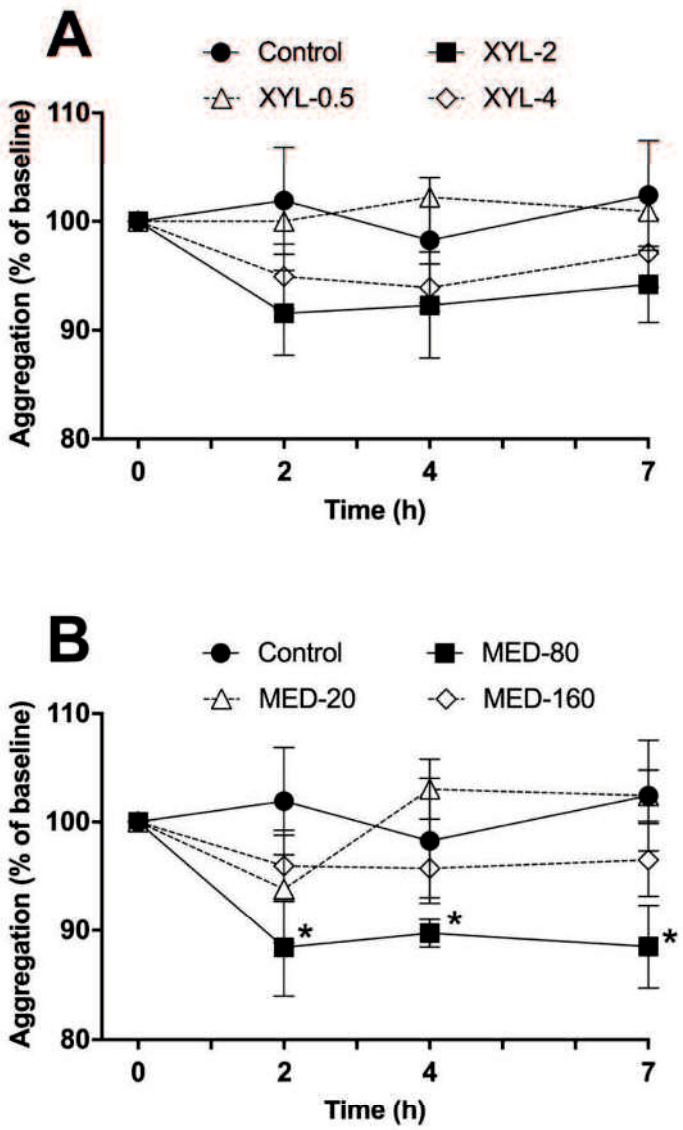

Figure 10. Mean ± standard error (SE) percentage changes of ex vivo platelet aggregation induced by 5.0 µmol/L adenosine diphosphate (ADP) after an intramuscular administration of xylazine (A) and medetomidine (B) in 5 cats. Values are reported as a percentage of the value for the 0 h (percentage aggregation of ADP at 0 h was assigned a value of 100%). *Value differs significantly ($P < 0.05$) from the value for the 0 h. See Figure 9 for remainder of key.

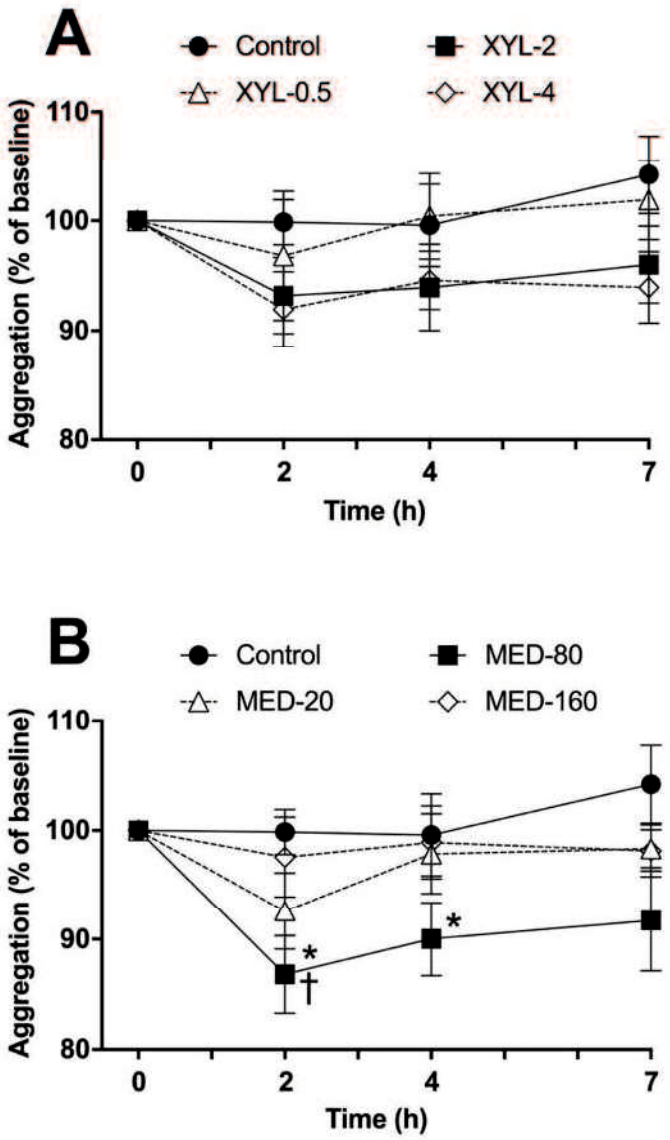

Figure 11 Mean ± standard error (SE) percentage changes of ex vivo platelet aggregation induced by 3.0 µg/mL collagen after an intramuscular administration of xylazine (A) and medetomidine (B) in 5 cats. Values are reported as a percentage of the value for the 0 h (percentage aggregation of collagen at 0 h was assigned a value of 100%). *Value differs significantly ($P < 0.05$) from the value for the 0 h. †Value differs significantly ($P < 0.05$) from the value for the control group. See Figure 9 for remainder of key.

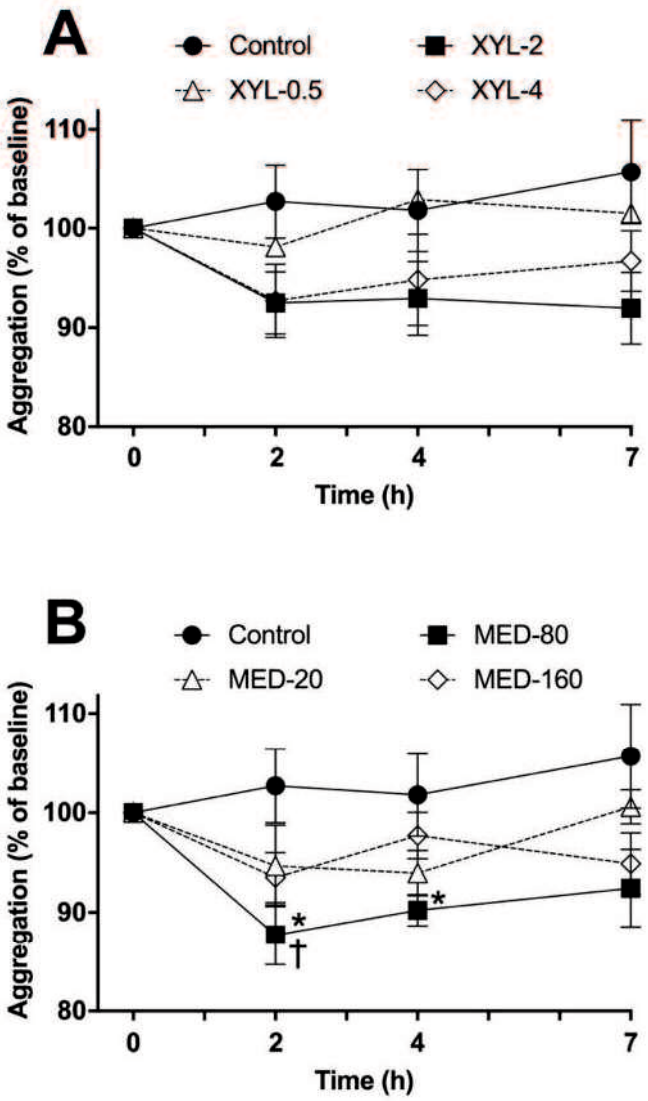

Figure 12. Mean ± standard error (SE) percentage changes of ex vivo platelet aggregation induced by 5.0 µg/mL collagen after an intramuscular administration of xylazine (A) and medetomidine (B) in 5 cats. Values are reported as a percentage of the value for the 0 h (percentage aggregation of collagen at 0 h was assigned a value of 100%). *Value differs significantly ($P < 0.05$) from the value for the 0 h. †Value differs significantly ($P < 0.05$) from the value for the control group. See Figure 9 for remainder of key.

Discussion

The results of this study demonstrated that an IM administration of 80 μg/kg MED inhibited ex vivo platelet aggregation, induced by both 3–5 μmol/L ADP and 3–5 μg/mL collagen in healthy cats; however, the inhibitory effects of MED were not dose-dependent within the tested doses. In addition, this study revealed that IM administrations of 0.5–4 mg/kg XYL did not significantly affect *ex vivo* platelet aggregation induced by both ADP and collagen. To the best of our knowledge, these findings are the first report outlining the *ex vivo* platelet aggregatory responses in cats that received MED and XYL systemically, and this is the first study comparing these 2 drugs.

These differences in platelet responses between MED and XYL administrations may be due to differences in receptor selectivity and specificity between the 2 drugs. In a previous *in vitro* study, it was reported that MED inhibited adrenaline-potentiated aggregation induced by ADP or collagen, whereas XYL was ineffective in inhibiting the adrenaline-potentiated aggregation in feline platelets.[36] It has been demonstrated that both feline and canine platelets have nonadrenergic I_1-receptors labeled by tritiated clonidine and I_2-receptors that had been labeled by tritiated idazoxan as well as α_2-adrenoceptors.[24] Furthermore, the affinities of MED to canine platelet I_1- and I_2-receptors have been reported to be approximately 16- and 55-fold, respectively, greater than those of XYL,[24] although in cats the affinities of both drugs to platelet I_1- and I_2-receptors have not been reported. A comparative study of the effects of I α-adrenergic agents on intraplatelet cyclic adenosine monophosphate (cAMP) and thromboxane B_2 (TXB_2) in canine and leporine platelets suggested that I α_2-adrenergic agents suppress cAMP production via the α_2-adrenoceptor, while exerting a negative control on TXB_2 generation via the arachidonic acid–thromboxane A_2 pathway.[77] In addition, a previous study has demonstrated that α_2-adrenoceptors

are expressed on canine, and feline platelets but not on bovine and equine platelets, and that all of 4 animal species platelets have both I_1- and I_2-receptor subtypes.[24] It has been also reported that bovine and equine platelets are unresponsive to catecholamines, but certain I α-adrenergic agents inhibit bovine and equine platelet aggregation induced by ADP or collagen.[76] These findings suggest that I α-adrenergic agents can inhibit platelet aggregation via non-adrenergic receptors including I-receptors. Therefore, the decrease of *ex vivo* ADP and collagen-induced platelet aggregation by an administration of 80 μg/kg MED in this study may be due to inhibiting platelet aggregation via the nonadrenoceptor binding sites including I_1- and I_2-receptors on feline platelets.

On the other hand, in the present study, 4 mg/kg XYL and 20 μg/kg MED insignificantly tended to reduce *ex vivo* collagen- or ADP-induced platelet aggregation at 2 h after administration. Both adrenaline and noradrenaline are reported to enhance the platelet aggregation induced by other stimulants, including ADP and collagen in cats.[22,36] Administrations of MED and XYL at the tested dosages in this study have been reported to reduce plasma concentrations of adrenaline and noradrenaline in cats.[27] These reports suggest that the decrease or the declining trend of platelet aggregation following administration of MED and XYL in this study may be partially related to the inhibition of endogenous catecholamine secretion via α_2-adrenoceptors, accompanied with the sedative effects of both agents. However, this reason alone cannot explain the difference in the effects of MED and XYL on ex vivo platelet aggregation.

In the present study, in cats, IM administration of MED at 20–160 μg/kg did not inhibit dose-dependent platelet aggregation induced by ADP and collagen. Similar dose-independent effects at higher doses (160 μg/kg) of MED have been also reported on hyperglycemia,

hypocatecholaminemia, and diuresis induced by MED in cats[27,42] and dogs.[2,67] Clonidine and related I drugs may be able to not only inhibit noradrenaline release in rat cerebral cortex through the α_2-adrenoceptor-mediated mechanism, but they may also induce a paradoxical noradrenaline release through an indirect mechanism related to a functional activity on I-receptors.[37] It has been also shown that noradrenaline release is reduced by I_1-receptors in addition to α_2-adrenoceptors in pithed hypertensive rats[55] while I_2-receptor selective ligands elevated extrasynaptic noradrenaline release in a rat brain microdialysis study.[1] These results suggest that I α_2-adrenergic agents at higher concentrations exert complex effects on catecholamine secretion via α_2-adrenoceptors, I_1-receptors, and I_2-receptors. The precise mechanisms by which the higher doses of MED do not further reduce platelet aggregation are not clear. However, as MED has an affinity for both I_1- and I_2-receptors on feline platelets[24] the dose-independency of MED on the inhibitory effect of platelet aggregation may be due to the complicated effects via the I_1- and I_2-receptors or other I-receptor subtypes at a higher concentration of MED.

Both XYL and MED are often used for sedation and analgesia and as a premedication for general anesthesia. The results in this study indicated that XYL may be used in cats with minimal concern for adverse effects on platelet function and hemostasis, because in clinical use, administrations of XYL at recommended doses (0.5–2 mg/kg) do not significantly affect platelet aggregation. However, the use of MED at a limited dose (80 μg/kg) may have inhibitory effects on feline platelet aggregation during certain events such as blood vessel damage and collagen exposure. On the other hand, it has been suggested that the sympathetic overactivity and hypercatecholaminemia may influence hemostasis via actions on platelets, coagulation and fibrinolytic factors, and endogenous anticoagulants.[62] In cats, hypercatecholaminemia occurs in conditions such as pheochromocytoma[72] endotoxin shock[17] and acute stress.[56] Fatal

thromboembolism was reported in a cat with pheochromocytoma.[10] A low-dose endotoxin infusion induces platelet aggregation[14] and intravascular coagulation is manifested during endotoxin shock in cats.[34] Therefore, the results of the study reported here suggest that MED may have clinical benefits for the hypercoagulatory state with hypercatecholaminemia, because catecholamines have a stimulatory effect on feline platelet aggregation.[36] However, further studies will be required to examine the effects of MED on platelet aggregation under various pathological conditions in cats.

In conclusion, administration of MED at 80 μg/kg reduced ex vivo platelet aggregation induced by both ADP and collagen compared with the control in this study. Administrations of XYL and MED at other dosages did not significantly affect the aggregation induced by ADP and collagen. Administrations of 4 mg/kg XYL and 20 μg/kg MED insignificantly tended to reduce ex vivo collagen- or ADP-induced platelet aggregation. These findings may be partially related to the inhibition of endogenous catecholamine secretion via α_2-adrenoceptors, accompanied with the sedative effects of both agents. However, the difference in the effects of MED and XYL on ex vivo platelet aggregation could not be explained only by the inhibition of catecholamine secretion. The present results suggest that the I structure, in part, plays a role in the inhibition of platelet aggregation. It was also found that the MED-induced inhibition of platelet aggregation was not dose-dependent in cats. These results indicated that systemic administration of XYL could be used in feline practice without concern for adverse effects on platelet function, although if MED is used, even in limited dosages, it may inhibit platelet aggregation.

General Conclusion

In chapter 1, the effects of various imidazoline or nonimidazoline α-adrenergic agents on *in vitro* aggregation and antiaggregation of feline platelets were examined. Both adrenaline and noradrenaline potentiated in a dose-dependent manner aggregation of feline platelets induced by ADP or collagen, but other α-adrenoceptor agonists, except for oxymetazoline and xylometazoline, did not potentiate platelet aggregation induced by ADP. Furthermore, results indicated that the α_2-adrenoceptor antagonists or certain imidazoline α-adrenergic agents (or both) inhibited, in a dose-dependent manner, adrenaline-potentiated aggregation induced by ADP or collagen, whereas α_1-adrenoceptor antagonists and nonimidazoline α-adrenergic agents were ineffective or less effective at inhibiting adrenaline-potentiated aggregation and that the α_2-adrenoceptor agonists medetomidine and xylazine did not reverse the inhibitory effects of the α_2-adrenoceptor antagonists atipamezole and yohimbine on adrenaline-potentiated aggregation. Furthermore, the results suggested that adrenaline-potentiated aggregation was mediated by α_2-adrenoceptors, whereas imidazoline agents inhibited platelet aggregation via imidazoline receptors in cats. Results of the present study also suggested that clinically recommended doses of xylazine may be used in feline practice with minimal concern for adverse effects on platelet function, although further *in vivo* or *ex vivo* studies will be required to examine the effects of these agents on platelet aggregation.

In chapter 2, the effects of medetomidine (20, 80, and 160 μg/kg) and xylazine (0.5, 2.0, and 4.0 mg/kg) administered intramuscularly (IM) on *ex vivo* platelet aggregation were compared in healthy cats. Administration of medetomidine at 80 μg/kg reduced *ex vivo* platelet aggregation

induced by both ADP and collagen compared with the control in this study. Administrations of xylazine and medetomidine at other dosages did not significantly affect the aggregation induced by ADP and collagen. Administrations of 4 mg/kg xylazine and 20 μg/kg medetomidine insignificantly tended to reduce ex vivo collagen- or ADP-induced platelet aggregation. These findings may be partially related to the inhibition of endogenous catecholamine secretion via α_2-adrenoceptors, accompanied with the sedative effects of both agents. However, the difference in the effects of medetomidine and xylazine on *ex vivo* platelet aggregation could not be explained only by the inhibition of catecholamine secretion. The present results suggest that the I structure, in part, plays a role in the inhibition of platelet aggregation. It was also found that the medetomidine-induced inhibition of platelet aggregation was not dose-dependent in cats. These results indicated that systemic administration of xylazine could be used in feline practice without concern for adverse effects on platelet function, although if medetomidine is used, even in limited dosages, it may inhibit platelet aggregation.

Abstract

In chapter 1, the study was aimed to investigate the effects of imidazoline or nonimidazoline α-adrenergic agents on aggregation of feline platelets. Blood obtained from 12 healthy adult cats. In 7 experiments, the effects of 23 imidazoline and nonimidazoline α-adrenoceptor agonists or antagonists on aggregation and antiaggregation of feline platelets were determined via a turbidimetric method. Collagen and ADP were used to initiate aggregation. Platelet aggregation was not induced by α-adrenoceptor agonists alone. Adrenaline and noradrenaline induced a dose-dependent potentiation of ADP- or collagen-induced aggregation. Oxymetazoline and xylometazoline also induced a small potentiation of ADP-stimulated aggregation, but other α-adrenoceptor agonists did not induce potentiation. The α_2-adrenoceptor antagonists or certain imidazoline α-adrenergic agents including phentolamine, yohimbine, atipamezole, clonidine, medetomidine, and dexmedetomidine inhibited adrenaline-potentiated aggregation induced by ADP or collagen in a dose-dependent manner. The imidazoline compound antazoline inhibited adrenaline-potentiated aggregation in a dose-dependent manner. Conversely, α_1-adrenoceptor antagonists and nonimidazoline α-adrenergic agents including xylazine and prazosin were ineffective or less effective for inhibiting adrenaline-potentiated aggregation. Moxonidine also was ineffective for inhibiting adrenaline-potentiated aggregation induced by collagen. Medetomidine and xylazine did not reverse the inhibitory effect of atipamezole and yohimbine on adrenaline-potentiated aggregation. In conclusion, adrenaline-potentiated aggregation in feline platelets may be mediated by α_2-adrenoceptors, whereas imidazoline agents may inhibit in vitro platelet aggregation via imidazoline receptors. Imidazoline α-adrenergic agents may have

clinical use for conditions in which there is platelet reactivity to adrenaline. Doses of xylazine may be used clinically in cats with minimal concerns for adverse effects on platelet function.

In chapter 2, this study aimed to investigate and compare the effects of systemic administration of medetomidine and xylazine on *ex vivo* platelet aggregation in clinically normal cats. Five cats were repeatedly used in each of 7 groups. The cats received saline as the nonmedicated control; 0.5, 2.0, and 4.0 mg/kg xylazine; and 20, 80, and 160 μg/kg body weight medetomidine intramuscularly. Venous blood was collected 4 times (0, 2, 4, and 7 h) after injection of both agents, and platelet-rich plasma was prepared. *Ex vivo* percent platelet aggregation was determined via a turbidimetric method. Collagen and ADP were used to initiate aggregation. Administrations of xylazine at all dosages did not significantly change the platelet aggregation induced by ADP and collagen compared with the control. In contrast, administration of medetomidine at 80 μg/kg significantly reduced platelet aggregation induced by both ADP and collagen compared with the control, whereas medetomidine at the other dosages did not significantly change the platelet aggregation induced by ADP and collagen. These results indicate that systemic administration of xylazine can be used in feline practice without concern for adverse effects on platelet function, although if medetomidine is used, even in limited dosages, it may inhibit platelet aggregation.

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