L-arginine and Arginase Products Potentiate Dexmedetomidine-induced Contractions in the Rat Aorta

Emily S. W. Wong, Ph.D., Ricky Y. K. Man, Ph.D., Kwok F. J. Ng, M.D., F.A.N.Z.C.A., Susan W. S. Leung, Ph.D., Paul M. Vanhoutte, M.D., Ph.D.

ABSTRACT

Background: The α_2-adrenergic sedative/anesthetic agent dexmedetomidine exerts biphasic effects on isolated arteries, causing endothelium-dependent relaxations at concentrations at or below 30 nM, followed by contractions at higher concentrations. L-arginine is a common substrate of endothelial nitric oxide synthase and arginases. This study was designed to investigate the role of L-arginine in modulating the overall vascular response to dexmedetomidine.

Methods: Isometric tension was measured in isolated aortic rings of Sprague Dawley rats. Cumulative concentrations of dexmedetomidine (10 nM to 10 μM) were added to quiescent rings (with and without endothelium) after previous incubation with vehicle, N^{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME; nitric oxide synthase inhibitor), prazosin (α_1-adrenergic antagonist), rauwolscine (α_2-adrenergic antagonist), L-arginine, (S)-(2-boronethyl)-L-cysteine hydrochloride (arginase inhibitor), N^{G}-hydroxy-L-arginine (arginase inhibitor), urea and/or ornithine. In some preparations, immunofluorescent staining, immunoblotting, or measurement of urea content were performed.

Results: Dexmedetomidine did not contract control rings with endothelium but evoked concentration-dependent increases in tension in such rings treated with L-NAME (E_{max} 50 ± 4%) or after endothelium-removal (E_{max} 74 ± 5%; N = 7 to 12). Exogenous L-arginine augmented the dexmedetomidine-induced contractions in the presence of L-NAME (E_{max} 75 ± 3%). This potentiation was abolished by (S)-(2-boronethyl)-L-cysteine hydrochloride (E_{max} 16 ± 4%) and N^{G}-hydroxy-L-arginine (E_{max} 18 ± 4%). Either urea or ornithine, the downstream arginase products, had a similar potentiating effect as L-arginine. Immunoassay measurements demonstrated an upregulation of arginase I by L-arginine treatment in the presence of L-NAME (N = 4).

Conclusions: These results suggest that when vascular nitric oxide homeostasis is impaired, the potentiation of the vasoconstrictor effect of dexmedetomidine by L-arginine depends on arginase activity and the production of urea and ornithine.

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DEXMEDETOMIDINE is an α_2-adrenoceptor agonist used as an intravenous sedative/anesthetic agent.[1] The popularity of the compound is based primarily on its bradycardic and sympatholytic effect when used clinically.[2,3] Besides causing sedation and anesthesia, dexmedetomidine also possesses clinically significant vascular effects; the systolic arterial blood pressure response to dexmedetomidine in patients can be quite different from that observed in healthy subjects—where most studies on the hemodynamic effects of the drug have been performed[4,5]—and can be unpredictable, with, for example, an increase during general anesthesia, but a decrease during regional anesthesia.[6] *In vivo*, the compound protects the rat myocardium against ischemia/

What We Already Know about This Topic

- Previous studies have demonstrated that the α_2-adrenergic sedative/anesthetic agent dexmedetomidine exerts biphasic vascular responses. L-arginine is the common substrate of endothelial nitric oxide synthase and arginases.
- This study investigated the role of L-arginine in modulating the overall vascular response to dexmedetomidine.

What This Article Tells Us That Is New

- These results suggest that when vascular nitric oxide homeostasis is impaired, the potentiation of the vasoconstrictor effect of dexmedetomidine by L-arginine depends on arginase activity and the production of urea and ornithine.

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reperfusion injury.[7] *In vitro,* dexmedetomidine contracts rat aortae without endothelium[8] and potentiates the contraction to 40 mM KCl in human gastroepiploic and internal mammary arteries without endothelium.[9,10] Earlier work from the laboratory showed that in rat mesenteric arteries with endothelium, dexmedetomidine causes concentration-dependent, biphasic changes in tension with α_2-adrenergic relaxation followed by α_1-adrenergic endothelium-independent contraction; in aortic rings, the former effect of the compound is masked by the latter.[11] Indeed, the relaxation induced by dexmedetomidine is prevented by α_2-adrenergic antagonists, endothelium-dependent and mediated by nitric oxide, whereas the contractile phase is potentiated by the nitric oxide synthase inhibitor N^{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME) or by removal of the endothelium, but abolished by the α_1-adrenoceptor antagonist prazosin.[11] These findings suggest that under control conditions, the direct α_1-adrenergic effect of dexmedetomidine causing contraction of vascular smooth muscle is blunted by endothelium-derived nitric oxide.

L-arginine the precursor of endothelial nitric oxide,[12] can reverse impairments of nitric oxide-mediated endothelium-dependent relaxations.[13] Clinically, acute L-arginine supplementation can improve the release of nitric oxide in patients suffering from endothelial dysfunction.[14–16] Besides nitric oxide synthase, L-arginine is also a substrate for arginases, major enzymes of the urea cycle.[17] In patients with vascular diseases with upregulated arginase activity, there is an associated decrease in nitric oxide-mediated dilatation.[18–20]

The present study was designed to investigate the vascular effects of dexmedetomidine under conditions of combined endothelial impairment and L-arginine supplementation, and to examine the hypothesis that arginases play a role in the observed effects. The results help to better understand the vasoconstrictor effects of dexmedetomidine in patients with endothelial dysfunction, particularly in stressing how the absence of preexisting constrictor tone, combined with endothelial dysfunction and/or L-arginine supplementation, affects the vascular response to dexmedetomidine.

Materials and Methods

All investigations were approved by the Committee for the Use of Laboratory Animals for Teaching and Research in the University of Hong Kong (Hong Kong, China), in accordance with the Guide for the Care and Use of Laboratory Animals by the National Research Council of United States. Ten weeks-old male Sprague Dawley rats were kept in a temperature-controlled room (21 ± 1°C) with a 12 h light dark cycle. They had free access to standard laboratory chow (LabDiet 5053, USA) and tap water.

Tissue Isolation for Organ Chamber Studies

Rats were anesthetized intraperitoneally with pentobarbital sodium (70 mg/kg; Ganes Chemicals Inc., USA). The thoracic aortae were isolated and transferred to oxygenated Krebs-Henseleit buffer (control solution) of the following composition: 120 mM NaCl, 25 mM $NaHCO_3$, 5.5 mM glucose, 4.8 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM NaH_2PO_4, and 1.25 mM $CaCl_2$. The isolated blood vessels were dissected free of surrounding fat and connective tissue and cut into six to eight rings (2 to 3 mm in length) for isometric tension measurements. The ring segments were randomly assigned to different treatments and studied in parallel in conventional organ chambers. Each ring was exposed to a different treatment, and no single ring was exposed to more than one treatment.

In some aortae, the endothelium was removed by perfusion with 1 ml 0.5% Triton X-100 solution for 30 s before cutting the rings. Integrity of the smooth muscle layer after endothelial removal was confirmed by normal contractions to 60 mM KCl (data not shown).

Isometric Tension Measurement

The aortic rings were suspended in organ chambers filled with 5 ml control solution maintained at 37°C and aerated with 95% O_2 and 5% CO_2. Each ring was connected to a force transducer (AD Instruments, Australia) for isometric tension recording (PowerLab, AD Instruments). The rings were allowed to equilibrate for 1.5 h to reach their optimal resting tensions of approximately 2.5 g, as determined in preliminary experiments (data not shown).

After exposing the rings twice to 60 mM KCl to obtain a reference contraction, the presence or absence of endothelium was confirmed by adding 1 μM acetylcholine to rings contracted with 1 μM phenylephrine.[21] The endothelium was considered viable when 80% or more relaxation to acetylcholine was obtained while it was regarded to be removed when relaxations to the muscarinic agonist were abolished. After determining endothelial viability, the rings were washed thoroughly, incubated with vehicle or different compounds including L-NAME (100 μM; Sigma-Aldrich, USA), prazosin (α_1-adrenoceptor antagonist, 1 μM; Sigma-Aldrich), rauwolscine (α_2-adrenoceptor antagonist, 100 nM; Sigma-Aldrich), L-arginine (1 mM; Sigma-Aldrich), (S)-(2-boronethyl)-L-cysteine hydrochloride (BEC, arginase inhibitor, 10 μM; Calbiochem, Germany), N^G-hydroxy-L-arginine (L-NOHA, arginase inhibitor, 10 μM; Calbiochem), urea (100 μM; Sigma-Aldrich) and ornithine (100 μM; Sigma-Aldrich) for 40 min. Cumulative concentrations of dexmedetomidine (10 nM to 10 μM; Abbott Laboratories, USA) were added to quiescent rings; the concentration of dexmedetomidine was increased when the reaction to the previous one had stabilized for at least three minutes, or after ten minutes in the absence of (further) increase in tension in response to the compound. Contractions to dexmedetomidine were expressed as percentages of the reference contraction to 60 mM KCl. At the end of each experiment, the viability of vascular smooth muscle was confirmed by the endothelium-independent relaxation caused by sodium nitroprusside (10 μM; Sigma-Aldrich) during contractions to phenylephrine.

Immunofluorescent Staining

Frozen sections of rat aortae were fixed with cold acetone for 10 min and then washed with water. After fixation, the sections were blocked with normal blocking serum for 30 min, incubated overnight at 4°C, and then at 37°C for one hour, with antibodies against von Willebrand factor (vWF,1:50; Sigma-Aldrich), arginase I (1:50; Santa Cruz, USA) or arginase II (1:50; Santa Cruz) in diluting buffer (phosphate buffered saline [PBS] + 0.01% (v/v) Triton X-100, 0.01% (v/v) Tween 20 and 0.1% (w/v) bovine serum albumin [BSA]). After incubation, the sections were washed with 0.01% Triton X in PBS and incubated with Alexa fluor 594 anti-rabbit IgG (1:50; Invitrogen, USA) and Alexa fluor 488 anti-goat IgG (1:50; Invitrogen) for two hours in the dark at room temperature. Antifade reagent with 4',6-diamino-2-phenylindole dihydrochloride was added. The frozen sections were then examined under a fluorescence microscope (Eclipse TE300; Nikon, Japan).

Urea Release

Rat aortae were incubated with dexmedetomidine and other compounds for three hours, and the solution bathing the rat aortae was collected. Samples were assayed in duplicate. The amount of urea present was measured using a QuantiChrom Urea assay kit (DIUR-500), following the manufacturer's (BioAssay Systems, USA) instructions. For each assay, working reagent was prepared freshly by mixing equal volumes of kit reagents A and B. Fifty μl of the samples and standards in serial dilutions were injected into separate wells of a 96-well plate; 200 μl of working reagent were added to each well. The plate was then put in the dark at room temperature for 50 min. The concentration of urea was determined by quantifying the optical density of each well at 450 nm using a microplate reader (MRX, Dynex Technology, USA).

Immunoblotting

The aortae used for the urea assay were collected and cut into small pieces. The samples were homogenized in lysis buffer (0.02 M Tris-HCl, 1% Triton X-100, 0.15 M NaCl, 1 mM ethylenediamine tetraacetic acid, 1 mM ethylene glycol tetraacetic acid, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM sodium orthovanadate) with 1:1,000 protease inhibitors cocktail. The mixture was centrifuged at 5,000 rpm for five minutes at 4°C. The supernatant was kept at –80°C until use. The protein concentrations of samples were measured using the Bradford assay. Protein samples were mixed with sodium dodecyl sulfate sample buffer and 5% bond breaker. The sample was boiled at 95°C for 10 min and subjected to 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were transferred electrophoretically onto nitrocellulose membranes. The membranes were blocked with 0.1% Tween Tris-buffered saline (TTBS) containing 2% BSA for two hours at room temperature and incubated overnight with primary antibodies (1:300 arginase I or II) in 2% BSA TTBS at 4°C. They then were washed three times for 10 min in TTBS. The membranes were incubated with secondary anti-goat antibodies (1:3,000; Dako, Denmark) at room temperature for two hours, followed by three more washes with TTBS. The probed membranes were visualized by chemiluminescence using an ECL plus Western Detection System (GE Healthcare Life Science, USA), and subsequently exposed to X-ray film (Fuji Photo Film, Germany). To reprobe β-actin, membranes were stripped with TTBS (pH 2) for five minutes. After washing thoroughly, they were blocked and subsequently reprobed with monoclonal β-actin antibodies (1:3,000; Sigma-Aldrich). The optical densities of the protein bands were determined with the computerized program Multi-Analysis (Bio-Rad Laboratories, USA). Densitometric analysis was normalized to the immunoreactive β-actin band.

Data Analysis

Contractions to dexmedetomidine are expressed as percentage of the reference contraction to 60 mM KCl obtained at the start of the experiment. The results are shown as mean ± SD, with N representing the number of rats. No blinding method was applied during these experiments; the present experiments were performed in parallel on preparations from the same aorta, thus scientifically permitting correct evaluation of the results without the need for "blind" analysis. Due to the limited number of aortic segments harvested, all combinations of treatment could not be studied at the same time. Some treatments were stopped with a sample size of four when significant differences were obtained. For the treatments that had a more fundamental role in comparison, they were repeated in each experiment so that the responses within the same artery could be compared. In order to avoid selection of data, all data obtained were compared. No missing, lost, or selected data have been excluded from the results. Data were analyzed and curve fitting was performed using the statistical program GraphPad Prism version 5.01 (GraphPad Software Inc., USA). To compare the effects of different inhibitors on dexmedetomidine-induced contractions, areas under the curve, half maximal effective concentrations (EC_{50}), and maximal effects (E_{max}) were calculated using the statistical program GraphPad Prism version 5.01. Since no significant changes in EC_{50} to dexmedetomidine were observed (table 1), most of the data of the functional studies are shown as areas under the curve. Data were analyzed by Student's *t* test for paired observations, one-way analysis of variances (ANOVA) followed by the Newman-Keuls *post hoc* test for multiple comparison, or two-way ANOVA followed by the Bonferroni *post hoc* test. An interim analysis was employed, but no further adjustments were made for multiple comparisons. *P* values less than 0.05 were considered to indicate statistically significant differences.

Results

Organ Chamber Studies

Dexmedetomidine did not cause significant increases in tension in control quiescent aortic rings with endothelium

Table 1. Summary of Effects of Endothelium Removal, or Incubation (40 min) with L-NAME (100 μM), Prazosin (1 μM), Rauwolscine (100 nM), L-arginine (1 mM), BEC (10 μM), L-NOHA (10 μM), Urea (100 μM), and Ornithine (100 μM), Alone or in Combination, on Contractions to Increasing Concentrations (10 nM to 10 μM) of Dexmedetomidine

Figure	Treatment	$logEC_{50}$	E_{max}
1	Control (with endothelium)	–6.8 ± 0.5	2 ± 1
	L-NAME (with endothelium)	–6.6 ± 0.1	50 ± 4
	Control (without endothelium)	–6.8 ± 0.1	74 ± 5
	L-NAME (without endothelium)	–6.9 ± 0.1	101 ± 4
2	L-NAME + Prazosin	Not definable	
	L-NAME + Rauwolscine	–6.3 ± 0.3	53 ± 11
3	L-arginine	–6.1 ± 0.4	4 ± 2
	L-NAME + L-arginine	–6.7 ± 0.1	75 ± 3
	L-NAME + L-arginine + BEC	–6.7 ± 0.4	16 ± 4
	L-NAME + L-arginine + L-NOHA	–6.5 ± 0.4	18 ± 4
	L-arginine (without endothelium)	–7.1 ± 0.1	117 ± 3
	L-NAME + L-arginine (without endothelium)	–7.0 ± 0.0	108 ± 2
4	L-NAME + Urea	–6.9 ± 0.1	96 ± 5
	L-NAME + Ornithine	–6.9 ± 0.1	95 ± 4

The E_{max} is expressed as percentage of the reference contraction to 60 mM KCl. EC_{50} is the concentration needed to obtain half-maximal contraction.

Data are mean ± SD.

BEC = (S)-(2-boronethyl)- L-cysteine hydrochloride; L-NAME = N^{ω}-nitro-L-arginine methyl ester hydrochloride; L-NOHA = N^{G}-hydroxy- L-arginine.

(fig. 1, *left*). However, the compound evoked concentration-dependent contractions in rings with endothelium when treated with the nitric oxide synthase inhibitor L-NAME (100 μM; E_{max} 50 ± 4%, N = 11) or in preparations without endothelium (E_{max} 74 ± 5%, N = 12); the response was significantly larger in the latter (fig. 1; table 1). In rings without endothelium, the contractions were significantly larger than those observed in preparations with endothelium treated with L-NAME and were significantly augmented further by the nitric oxide synthase inhibitor (E_{max} 101 ± 4%; N = 13; fig. 1; table 1). In preparations with endothelium, treated with L-NAME, the contractions to dexmedetomidine were abolished by prazosin (1 μM), but not significantly affected by rauwolscine (100 nM; fig. 2; table 1).

In rings with endothelium, incubation with L-arginine (1 mM) did not unmask contractions to dexmedetomidine, but did significantly augment the concentration-dependent contractions to the compound in preparations treated with L-NAME (100 μM) (E_{max} 75 ± 3%, N = 4; fig. 3; table 1). The arginase inhibitors BEC (10 μM) and L-NOHA (10 μM) significantly attenuated the contraction to dexmedetomidine of rings with endothelium incubated with L-arginine plus L-NAME (BEC: E_{max} 16 ± 4%, N = 9 and L-NOHA: E_{max} 18 ± 4%, N = 5; fig. 3; table 1). In the absence of endothelium, incubation with L-arginine alone resulted in contractions significantly larger than those observed in rings treated with L-NAME; no further potentiation was observed after combined incubation with L-arginine plus L-NAME (fig. 3; table 1).

L-arginine is the common substrate for nitric oxide synthases and arginases. In the urea cycle, L-arginine is hydrolyzed by arginase to produce urea and ornithine.[22] The arginase products, urea (100 μM) and ornithine (100 μM) significantly augmented the concentration-dependent contractions to dexmedetomidine in the presence of L-NAME (fig. 4; table 1). The augmentation was similar to that obtained with L-arginine. The potentiating effects of urea or ornithine alone were not significantly different (fig. 4; table 1).

Immunofluorescent Staining

Arginase consists of two isoforms: arginase I and II.[23] Fluorescence staining revealed that in rat aortae, two isoforms of the enzyme are localized mainly in the endothelium, and are also present in the vascular smooth muscle cells (fig. 5).

Urea Production

To determine whether or not the potentiation caused by L-arginine on the contraction to dexmedetomidine is due to the synthesis of downstream products catalyzed by arginases, urea levels were determined in the solution bathing aortic rings exposed to dexmedetomidine (10 μM), alone or in combination with L-NAME and/or L-arginine for three hours; this duration of treatment is approximately equal to the time needed for the functional studies reported above. The release of urea increased significantly in aortic rings treated with L-NAME or L-arginine (fig. 6).

Immunoblotting

After measuring the urea production, the preparations were collected for Western blotting to determine the presence of arginase I and II. The two isoforms were present in the rat aortae. In the presence of dexmedetomidine (10 μM), the presence of arginase I was significantly enhanced in rings treated with L-NAME and L-arginine (fig. 7), while the levels of arginase II were reduced in rings incubated with L-NAME (fig. 7).

Discussion

The present experiments were performed on isolated rings of rat aortae and studied under isometric conditions. This is a standard procedure to pharmacologically evaluate endothelium-dependent and independent responses;[24–27] it permits the parallel study of several preparations from the same blood vessel, while exposing each preparation to only one concentration-response curve of dexmedetomidine, thus reducing biases due to interanimal variability or to sequential administration of the compound.

Dexmedetomidine is used clinically for sedation before and during surgical procedures, as well as in mechanically ventilated patients in the intensive care unit.[28] The peak plasma concentration of dexmedetomidine ranges between 0.71 and 1.71 ng/ml (approximately 3 to 7 nM) in patients requiring postoperative sedation in the intensive care unit,

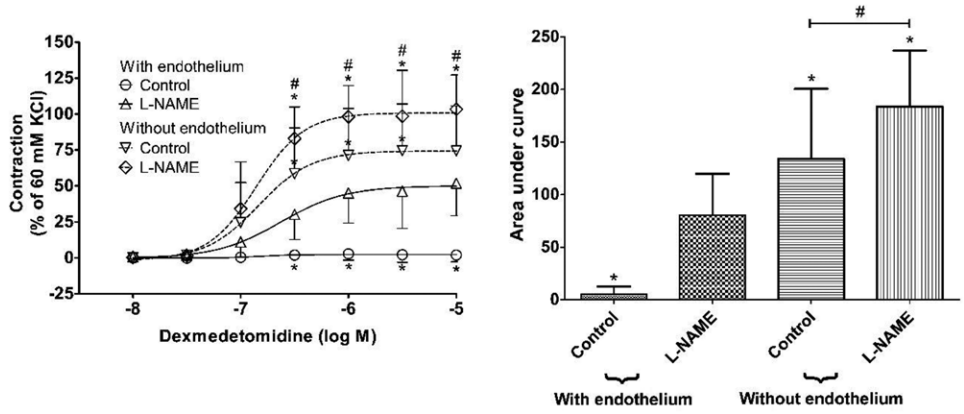

Fig. 1. Effect of endothelium-removal and incubation (40 min) with N$^{\omega}$-nitro- L-arginine methyl ester hydrochloride (L-NAME) on contractions to cumulatively increasing concentrations (10 nM to 10 μM) of dexmedetomidine in quiescent rat aortic rings. Preparations with (N = 7) and without endothelium (N = 12) and L-NAME (100 μM; with endothelium, N = 11, and without endothelium, N = 13) are shown. Data expressed as concentration-response curves and analyzed by two-way ANOVA followed by the Bonferroni *post hoc* test (*left*), and as areas under the curve and analyzed by one-way ANOVA followed by the Newman-Keuls Multiple Comparison *post hoc* test (*right*), and shown as mean ± SD. * and # indicate statistically significant differences ($P < 0.05$) with L-NAME (with endothelium) or between preparations without endothelium, respectively.

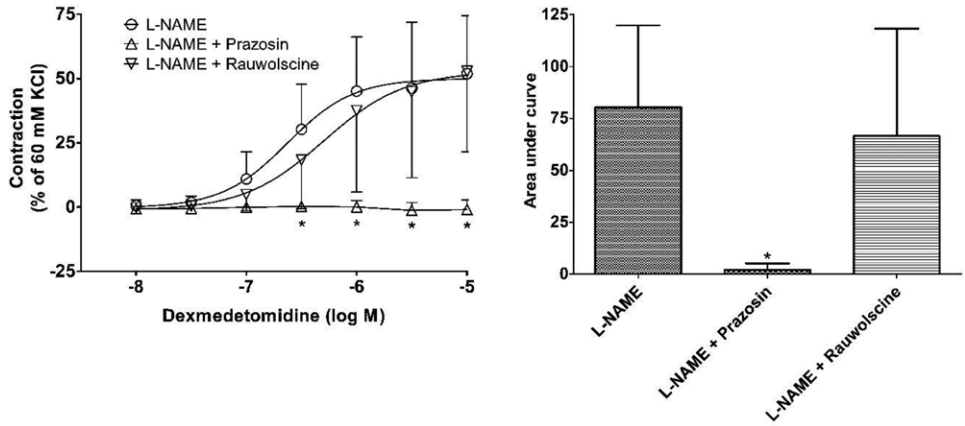

Fig. 2. Effect of incubation (40 min) with prazosin (1 μM; N = 6) or rauwolscine (100 nM; N = 6) on contractions to cumulatively increasing concentrations (10 nM to 10 μM) of dexmedetomidine in quiescent rat aortic rings incubated with N$^{\omega}$-nitro-L-arginine methyl ester hydrochloride (L-NAME; N = 11). Data expressed as concentration-response curves and analyzed by two-way ANOVA followed by the Bonferroni *post hoc* test (*left*), and as areas under the curve and analyzed by one-way ANOVA followed by the Newman-Keuls multiple comparison *post hoc* test (*right*), and shown as mean ± SD. * indicates statistically significant differences ($P < 0.05$) with preparations treated with L-NAME only.

and receiving a loading infusion of 2.5 μg/kg over 10 min followed by a maintenance infusion of 0.7 $\mu g \cdot kg^{-1} \cdot h^{-1}$.[29] With a dose of dexmedetomidine adjusted to maintain critically ill patients in the predefined target sedation range, the plasma concentrations of the drug vary greatly, from an undetectable level to more than 30 ng/ml (approximately 126 nM).[30,31] In this study, dexmedetomidine, at the clinically relevant concentration range (between 10 nM and 10 μM), induced a concentration-dependent contraction in quiescent isolated rat aortic rings, after endothelium-removal or L-NAME-treatment. These findings, in line with observations in either human or rat blood vessels,[8–11,32] show that absence (endothelium-removal) or impaired function of endothelial nitric oxide synthase (incubation with L-NAME) enhances the vasoconstrictor effect of dexmedetomidine. In the presence of prazosin, but not rauwolscine, the contractions to dexmedetomidine were abolished. These results differ from previous studies indicating that both α_1-adrenergic and α_2-adrenergic receptors contribute to dexmedetomidine-induced contraction in the mesenteric artery of the same species.[11] This can be explained by the differential distribution of the two adrenoceptor subtypes in individual vascular beds. Indeed, α_1-adrenergic receptors are dominant in the aorta[33] while the α_2-adrenergic subtype is more prominent in smaller arteries.[34]

An unexpected observation in present experiments was that the nitric oxide synthase inhibitor L-NAME[35] also augmented the contraction to dexmedetomidine in arteries

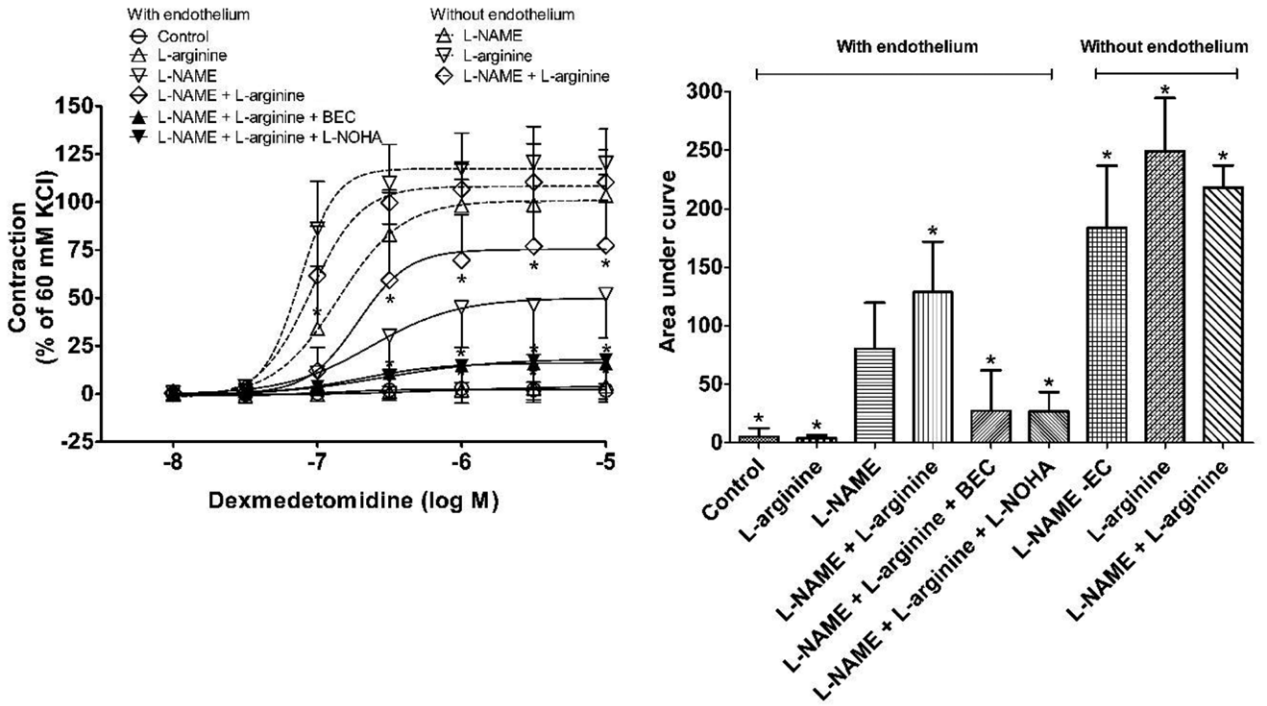

Fig. 3. Effect of incubation (40 min) with N$^{\omega}$-nitro-L-arginine methyl ester hydrochloride (L-NAME; 100 μM; N = 11), L-arginine (1 mM; N = 4), (S)-(2-boronethyl)-L-cysteine hydrochloride (BEC;100 μM; N = 9) or N^{G}-hydroxy-L-arginine (L-NOHA; 100 μM; N = 5), given alone or in combination (N = 15), on contractions to cumulatively increasing concentrations (10 nM to 10 μM) of dexmedetomidine in quiescent rat aortic rings with or without endothelium. Data expressed as concentration-response curves and analyzed by two-way ANOVA followed by the Bonferroni *post hoc* test (*left*), and as areas under the curve and analyzed by one-way ANOVA followed by the Newman-Keuls Multiple Comparison *post hoc* test (*right*), and shown as mean ± SD. **P* < 0.05 compared with L-NAME.

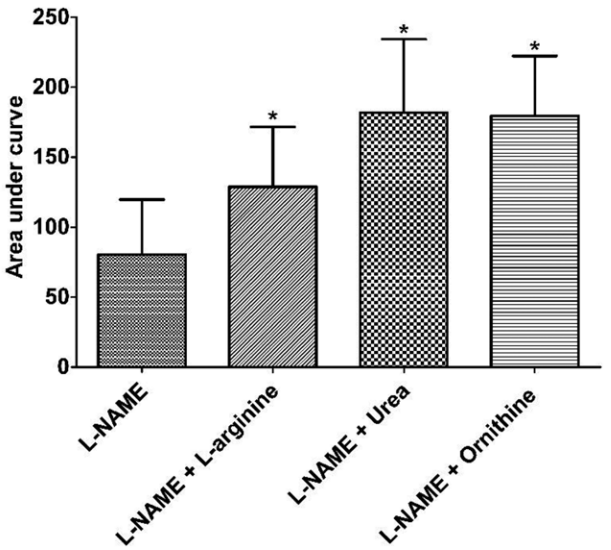

Fig. 4. Effect of incubation (40 min) with, L-arginine (1 mM; N = 15), urea (100 μM; N = 8) or ornithine (100 μM; N = 8) on contractions to cumulatively increasing concentrations (10 nM to 10 μM) of dexmedetomidine in quiescent rat aortic rings with endothelium. All preparations were incubated with N$^{\omega}$-nitro-L-arginine methyl ester hydrochloride (L-NAME; 100 μM). Data expressed as areas under the curve and analyzed by one-way ANOVA followed by the Newman-Keuls Multiple Comparison *post hoc* test, and shown as mean ± SD. **P* < 0.05 compared with L-NAME.

without endothelium. These results could indicate that the removal of the endothelium had not been complete, which is made unlikely by the disappearance of the relaxation induced by acetylcholine.[21] An alternative explanation is the presence of neuronal nitric oxide synthase in rat aortae,[36] the activity of which would also be inhibited by L-NAME. Moreover, endothelium removal potentiated dexmedetomidine-induced contractions more than nitric oxide synthase inhibition in rings with endothelium, confirming the endothelial nitric oxide synthase-independent production of nitric oxide by the endothelial cells of the rat aorta.[37] The present experiments were performed in a high P_{O_2} environment (95% O_2); if anything, this may underestimate the involvement of nitric oxide.[38,39]

L-arginine is the substrate for nitric oxide production by nitric oxide synthase.[12] Under most experimental conditions, the acute administration of L-arginine improves endothelial function, thus facilitating vasodilatation.[14–16,22] Exogenous L-arginine at 1 mM (the same concentration used for incubation in the present experiments), causes endothelium-independent relaxations of the rat aorta.[22] However, the present results demonstrate that L-arginine augments, rather than inhibits, dexmedetomidine-induced contraction in aortic rings incubated with L-NAME, under conditions where nitric oxide synthase is not likely to be operative.

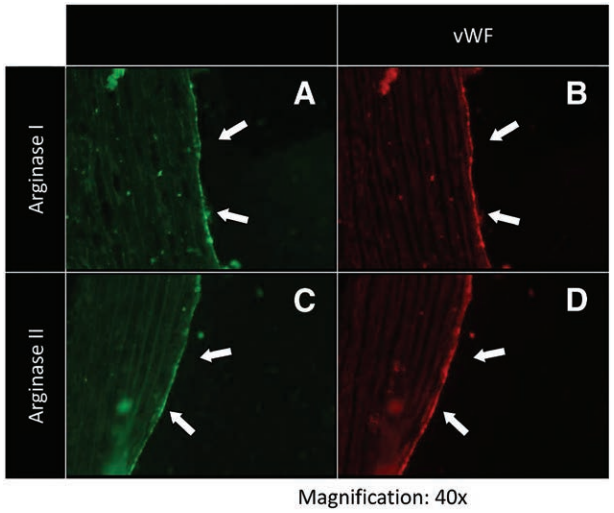

Fig. 5. Immunofluorescent staining with antibodies against arginase I, arginase II, or von Willebrand factor (vWF) in frozen sections of rat aorta with endothelium. The green fluorescence (*arrows*) in panel *A* indicates the presence of arginase I and in panel *C* that of arginase II. The red fluorescence (*arrows*) in *B* and *D* shows vWF as an endothelial cell marker.

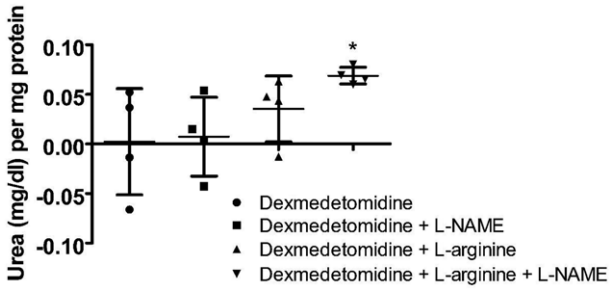

Fig. 6. Quantitative colorimetric determination of urea production in the solution bathing rat aortae with endothelium after incubation (3 h, to mimic the total duration of the functional experiments) with various agents. Data were normalized to mg protein and analyzed by Student's *t* test and are shown as a scatter plot with mean ± SD. * indicates statistically significant differences ($P < 0.05$) with dexmedetomidine + N^{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME) and between dexmedetomidine + L-arginine; N = 4. Dexmedetomidine (10 μM), L-NAME (100 μM), L-arginine (1 mM).

L-arginine is a common substrate for nitric oxide synthase and arginase; the latter catalyzes the hydrolysis of L-arginine into urea and ornithine.[40] Both arginase I and II are present in rat aortae,[41] as confirmed by the present Western blotting data. In this study, the increase in dexmedetomidine-induced contraction caused by L-arginine in aortae treated with L-NAME was abolished by the arginase inhibitors BEC and L-NOHA.[42] Although the use of L-NOHA as an inhibitor of arginase is complicated by the fact that it is also a precursor for nitric oxide synthesis,[40] the similarity of the inhibition observed with BEC permits the conclusion that arginase is involved in the potentiation of the response to dexmedetomidine by L-arginine. Not only did BEC and L-NOHA reverse the L-arginine potentiation, but they also reduced the contraction to dexmedetomidine in the presence of L-NAME. BEC and L-NOHA are competitive inhibitors that do not inhibit nitric oxide synthase at concentrations that inhibit arginases.[40] However, they reverse tolerance to acetylcholine, indicating an augmentation in nitric oxide bioavailability.[42] Moreover, the increase in dexmedetomidine-induced contraction by L-arginine was observed only in preparations with endothelium. This concurs with the present immunofluorescent staining results that showed the presence of the two isoforms, arginase I and II, mainly in the endothelial cells of the rat aorta, although they have been reported by others to also be present in vascular smooth muscle cells.[17,40] The specific subtype of arginase responsible for enhancing the contraction to dexmedetomidine could not be determined as isoform-specific arginase inhibitors are not available. However, the changes in protein presence of the two isoforms observed in the present experiments suggests a major role of arginase I. Although the protein level of arginase II was more prominent than that of arginase I, in rings treated with L-NAME, the level of arginase I was augmented, while that of arginase II was diminished. When incubated with L-NAME and L-arginine the presence of arginase I increased while that of arginase II remained unchanged. Taken into consideration together, these results suggest that the potentiation of contraction to dexmedetomidine by L-arginine in the presence of L-NAME is likely due to the up-regulation of arginase I.

As mentioned in the previous paragraph, urea and ornithine are the major products generated by arginase.[40] They do not alter nitric oxide production[43] and urea does not reverse the inhibition of endothelium-dependent relaxation caused by nitric oxide synthase inhibition.[44] The metabolism of L-arginine by arginases produces equal amounts of urea and ornithine.[45] Hence, the same concentration (100 μM) of the two arginase products was used in the present study.[13,44] Higher concentrations were not considered in order to prevent a potential inhibitory effect on arginases.[46] The two arginase products augmented dexmedetomidine-induced contraction to a comparable extent and shared similar potentiating effects with L-arginine . At least in regards to urea, the endothelial cells of the rat aorta abundantly contain the urea transporter-B permitting its intracellular uptake.[47] The current findings do not permit further speculation concerning the mechanism underlying the augmentation of the contractions to dexmedetomidine caused by the arginase products. However, the abundancy of arginases in endothelial cells and the strict endothelium-dependency of the response to L-arginine suggest the release of endothelium-derived contracting factors (*e.g.,* vasoconstrictor prostanoids or endothelin-1, the release of which is potentiated when endothelial nitric oxide synthase is dysfunctional[20]) diffusing to the vascular smooth muscle cells and reinforcing the α_1-adrenergic activation caused by the compound. Further investigations are warranted to verify this interpretation.

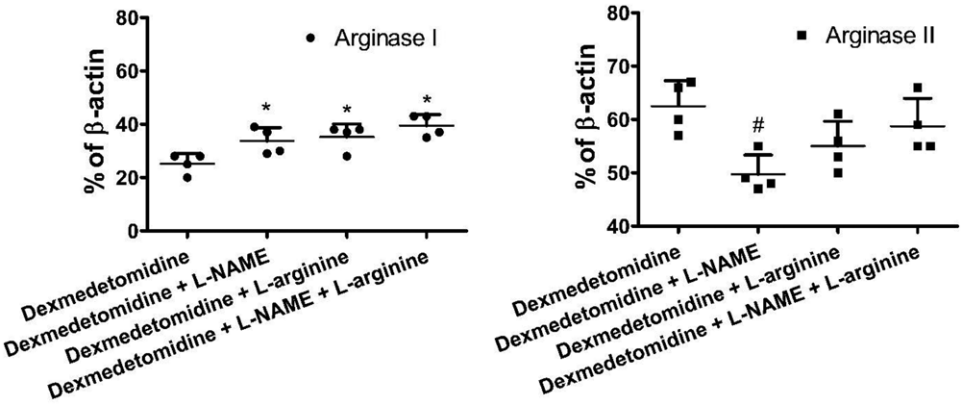

Fig. 7. Protein presence (measured by Western blotting) of arginase I (*left*) and II (*right*) in rat aortae with endothelium. Aortic rings were incubated with dexmedetomidine (10 μM), Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME; 100 μM) or L-arginine (1 mM) for three hours. Average data were normalized to β-actin and analyzed by one-way ANOVA followed by the Newman-Keuls Multiple Comparison *post hoc* test, and are shown as scatter plots with mean ± SD. * and # represent $P < 0.05$ in corresponding arginase expression *versus* dexmedetomidine alone; N = 4.

In any case, the present results suggest that the increase in exogenous L-arginine supply, combined with the blockade of nitric oxide synthase by L-NAME which favors arginases, stimulates the activity of the latter, and therefore increases the endogenous production of urea (and ornithine). This interpretation is supported by the present measurements using a quantitative colorimetric urea assay, under conditions mimicking sustained infusions of dexmedetomidine in patients.[29,30] In the presence of the compound, the level of urea was augmented in aortae incubated with L-arginine and L-NAME, compared with those treated with L-NAME only. Ornithine formation was not measured in the present experiments. Nevertheless, increased generation of urea and ornithine, the downstream products of the transformation of L-arginine by arginase, can explain the potentiating effects of L-arginine on dexmedetomidine-induced contraction when endothelial nitric oxide synthase is dysfunctional due to the presence of L-NAME (fig. 8).

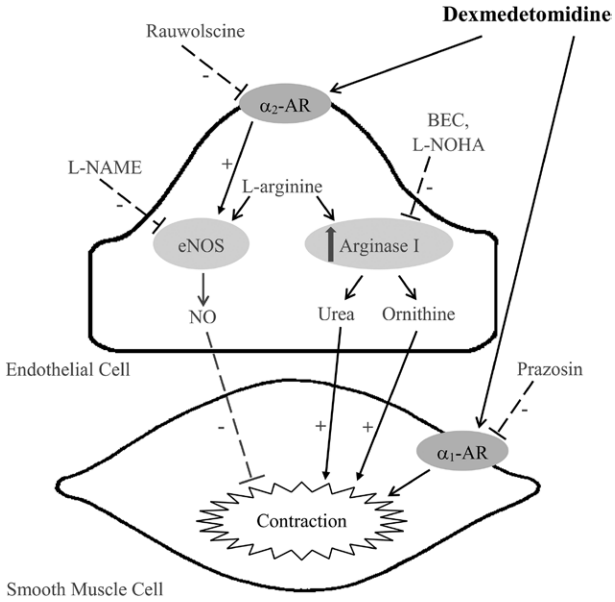

Fig. 8. Schematic summarizing the findings of the study. In the rat aorta, dexmedetomidine activates α_1-adrenergic receptors (AR), which can be inhibited by prazosin, on smooth muscle cells to cause contraction; the contraction is likely counteracted by activation of endothelial α_2-AR, which can be inhibited by rauwolscine, and are coupled to endothelial nitric oxide synthase (eNOS) leading to production of nitric oxide (NO), a major endothelium-derived relaxing factor. Therefore, the contraction is unmasked by the eNOS inhibitor Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME), which results not only in inhibition of NO production, but also in a shift of the substrate L-arginine to metabolism by arginase I, which can be inhibited by (S)-(2-boronethyl)- L-cysteine hydrochloride (BEC) and NG-hydroxy-L-arginine (L-NOHA), to urea and ornithine. L-arginine, together with dexmedetomidine, appears to promote the upregulation of arginase I, leading to further increase in the production of urea (and presumably that of ornithine), which contributes to the potentiation of the α_1-AR-mediated contraction induced by dexmedetomidine. + = activation; - = inhibition.

Conclusions

In terms of clinical relevance, the contractions observed in quiescent arteries that lack endothelium or have impaired nitric oxide synthase activity suggest that the effect of dexmedetomidine on systemic vascular resistance and blood pressure will depend on the functional state of the endothelium. When using dexmedetomidine, the main challenge is to avoid profound hypotension. Thus, the present study may provide an explanation for the clinical observation that dexmedetomidine seems well-tolerated in patients in whom endothelial function is impaired since the present results indicate that such dysfunction favors the vasoconstrictor effects of the compound.[48]

Patients with endothelial dysfunction may receive acute L-arginine supplementation to improve their endothelial function.[14,15] However, the results of this study demonstrate that L-arginine may amplify the vasoconstrictor potential of

dexmedetomidine. This amplification by L-arginine depends on the activity of arginase, with the resulting production of urea and ornithine. Therefore, caution may be required when dexmedetomidine is administered to patients with endothelial dysfunction and impaired vascular nitric oxide homeostasis. This could be of particular importance in arteries lined with regenerated endothelial cells, which selectively lose the beneficial activation of endothelial nitric oxide synthase due to stimulation of G_i-coupled cell membrane receptors, among which the α_2-adrenergic receptors activated by dexmedetomidine.[16,20,49] Vascular response data obtained with dexmedetomidine in healthy volunteers[50] may predict poorly the actual response in such patients. Further *in vivo* studies and clinical trials are required to demonstrate how coexisting conditions (*e.g.,* intake of medications) that may affect the activities of endothelial nitric oxide synthase and/or arginases can affect responses to dexmedetomidine in patients.

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Competing Interests

The authors declare no competing interests.

Correspondence

Address correspondence to Dr. Leung: 2/F, Laboratory Block, Li Ka Shing Faculty of Medicine, 21 Sassoon Road, Pokfulam, Hong Kong, China. swsleung@hku.hk. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

References

1. Bhana N, Goa KL, McClellan KJ: Dexmedetomidine. Drugs 2000; 59:263–8; discussion 269–70
2. Afonso J, Reis F: Dexmedetomidine: Current role in anesthesia and intensive care. Rev Bras Anestesiol 2012; 62:118–33
3. Naaz S, Ozair E: Dexmedetomidine in current anaesthesia practice: A review. J Clin Diagn Res 2014; 8:GE01–4
4. Hogue CW Jr, Talke P, Stein PK, Richardson C, Domitrovich PP, Sessler DI: Autonomic nervous system responses during sedative infusions of dexmedetomidine. ANESTHESIOLOGY 2002; 97:592–8
5. Ehara T, Ogawa Y, Kato J, Aoki K, Ogawa S, Iwasaki K: The effect of dexmedetomidine on arterial-cardiac baroreflex function assessed by spectral and transfer function analysis. J Anesth 2012; 26:483–9
6. Talke P, Lobo E, Brown R: Systemically administered alpha2-agonist-induced peripheral vasoconstriction in humans. ANESTHESIOLOGY 2003; 99:65–70
7. Behmenburg F, Pickert E, Mathes A, Heinen A, Hollmann MW, Huhn R, Berger MM: The cardioprotective effect of dexmedetomidine in rats is dose-dependent and mediated by bkca channels. J Cardiovasc Pharmacol 2017; 69:228–35
8. Kim HJ, Sohn JT, Jeong YS, Cho MS, Kim HJ, Chang KC, Shin MK, Park CS, Chung YK: Direct effect of dexmedetomidine on rat isolated aorta involves endothelial nitric oxide synthesis and activation of the lipoxygenase pathway. Clin Exp Pharmacol Physiol 2009; 36:406–12
9. Hamasaki J, Tsuneyoshi I, Katai R, Hidaka T, Boyle WA, Kanmura Y: Dual alpha(2)-adrenergic agonist and alpha(1)-adrenergic antagonist actions of dexmedetomidine on human isolated endothelium-denuded gastroepiploic arteries. Anesth Analg 2002; 94:1434–40
10. Yildiz O, Ulusoy HB, Seyrek M, Gul H, Yildirim V: Dexmedetomidine produces dual alpha2-adrenergic agonist and alpha1-adrenergic antagonist actions on human isolated internal mammary artery. J Cardiothorac Vasc Anesth 2007; 21:696–700
11. Wong ES, Man RY, Vanhoutte PM, Ng KF: Dexmedetomidine induces both relaxations and contractions, via different {alpha}2-adrenoceptor subtypes, in the isolated mesenteric artery and aorta of the rat. J Pharmacol Exp Ther 2010; 335:659–64
12. Palmer RM, Ashton DS, Moncada S: Vascular endothelial cells synthesize nitric oxide from L-arginine. Nature 1988; 333:664–6
13. Moore PK, al-Swayeh OA, Chong NW, Evans RA, Gibson A: L-NG-nitro arginine (L-NOARG), a novel, L-arginine-reversible inhibitor of endothelium-dependent vasodilatation *in vitro*. Br J Pharmacol 1990; 99:408–12
14. Drexler H, Zeiher AM, Meinzer K, Just H: Correction of endothelial dysfunction in coronary microcirculation of hypercholesterolaemic patients by L-arginine. Lancet 1991; 338:1546–50
15. Gornik HL, Creager MA: Arginine and endothelial and vascular health. J Nutr 2004; 134(10 Suppl):2880S–2887S; discussion 2895S
16. Vanhoutte PM, Zhao Y, Xu A, Leung SW: Thirty Years of Saying NO: Sources, Fate, Actions, and Misfortunes of the Endothelium-Derived Vasodilator Mediator. Circ Res 2016; 119:375–96
17. Pernow J, Jung C: Arginase as a potential target in the treatment of cardiovascular disease: Reversal of arginine steal? Cardiovasc Res 2013; 98:334–43
18. Zhang C, Hein TW, Wang W, Miller MW, Fossum TW, McDonald MM, Humphrey JD, Kuo L: Upregulation of vascular arginase in hypertension decreases nitric oxide-mediated dilation of coronary arterioles. Hypertension 2004; 44:935–43
19. Ryoo S, Gupta G, Benjo A, Lim HK, Camara A, Sikka G, Lim HK, Sohi J, Santhanam L, Soucy K, Tuday E, Baraban E, Ilies M, Gerstenblith G, Nyhan D, Shoukas A, Christianson DW, Alp NJ, Champion HC, Huso D, Berkowitz DE: Endothelial arginase II: A novel target for the treatment of atherosclerosis. Circ Res 2008; 102:923–32
20. Vanhoutte PM, Shimokawa H, Feletou M, Tang EH: Endothelial dysfunction and vascular disease—a 30th anniversary update. Acta Physiol (Oxf) 2017; 219:22–96
21. Furchgott RF, Zawadzki JV: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980; 288:373–6
22. Schini VB, Vanhoutte PM: arginine evokes both endothelium-dependent and -independent relaxations in L-arginine-depleted aortas of the rat. Circ Res 1991; 68:209–16
23. Jenkinson CP, Grody WW, Cederbaum SD: Comparative properties of arginases. Comp Biochem Physiol B Biochem Mol Biol 1996; 114:107–32
24. Muldoon SM, Vanhoutte PM, Lorenz RR, Van Dyke RA: Venomotor changes caused by halothane acting on the sympathetic nerves. ANESTHESIOLOGY 1975; 43:41–8

25. De Mey JG, Vanhoutte PM: Anoxia and endothelium-dependent reactivity of the canine femoral artery. J Physiol 1983; 335:65–74
26. Gräser T, Vanhoutte PM: Hypoxic contraction of canine coronary arteries: role of endothelium and cGMP. Am J Physiol 1991; 261(6 Pt 2):H1769–77
27. Chan CK, Mak J, Gao Y, Man RY, Vanhoutte PM: Endothelium-derived NO, but not cyclic GMP, is required for hypoxic augmentation in isolated porcine coronary arteries. Am J Physiol Heart Circ Physiol 2011; 301:H2313–21
28. Carollo DS, Nossaman BD, Ramadhyani U: Dexmedetomidine: a review of clinical applications. Curr Opin Anaesthesiol 2008; 21:457–61
29. Venn RM, Karol MD, Grounds RM: Pharmacokinetics of dexmedetomidine infusions for sedation of postoperative patients requiring intensive caret. Br J Anaesth 2002; 88:669–75
30. Iirola T, Ihmsen H, Laitio R, Kentala E, Aantaa R, Kurvinen JP, Scheinin M, Schwilden H, Schüttler J, Olkkola KT: Population pharmacokinetics of dexmedetomidine during long-term sedation in intensive care patients. Br J Anaesth 2012; 108:460–8
31. Välitalo PA, Ahtola-Sätilä T, Wighton A, Sarapohja T, Pohjanjousi P, Garratt C: Population pharmacokinetics of dexmedetomidine in critically ill patients. Clin Drug Investig 2013; 33:579–87
32. Snapir A, Talke P, Posti J, Huiku M, Kentala E, Scheinin M: Effects of nitric oxide synthase inhibition on dexmedetomidine-induced vasoconstriction in healthy human volunteers. Br J Anaesth 2009; 102:38–46
33. Aboud R, Shafii M, Docherty JR: Investigation of the subtypes of alpha 1-adrenoceptor mediating contractions of rat aorta, vas deferens and spleen. Br J Pharmacol 1993; 109:80–7
34. Flavahan NA, Cooke JP, Shepherd JT, Vanhoutte PM: Human postjunctional alpha-1 and alpha-2 adrenoceptors: Differential distribution in arteries of the limbs. J Pharmacol Exp Ther 1987; 241:361–5
35. Rees DD, Palmer RM, Schulz R, Hodson HF, Moncada S: Characterization of three inhibitors of endothelial nitric oxide synthase *in vitro* and *in vivo*. Br J Pharmacol 1990; 101:746–52
36. Boulanger CM, Heymes C, Benessiano J, Geske RS, Lévy BI, Vanhoutte PM: Neuronal nitric oxide synthase is expressed in rat vascular smooth muscle cells: activation by angiotensin II in hypertension. Circ Res 1998; 83:1271–8
37. Zhao Y, Vanhoutte PM, Leung SW: Endothelial nitric oxide synthase-independent release of nitric oxide in the aorta of the spontaneously hypertensive rat. J Pharmacol Exp Ther 2013; 344:15–22
38. Takehara Y, Kanno T, Yoshioka T, Inoue M, Utsumi K: Oxygen-dependent regulation of mitochondrial energy metabolism by nitric oxide. Arch Biochem Biophys 1995; 323:27–32
39. Tsuchiya M, Tokai H, Takehara Y, Haraguchi Y, Asada A, Utsumi K, Inoue M: Interrelation between oxygen tension and nitric oxide in the respiratory system. Am J Respir Crit Care Med 2000; 162(4 Pt 1):1257–61
40. Morris SM Jr: Recent advances in arginine metabolism: roles and regulation of the arginases. Br J Pharmacol 2009; 157:922–30
41. Demougeot C, Prigent-Tessier A, Marie C, Berthelot A: Arginase inhibition reduces endothelial dysfunction and blood pressure rising in spontaneously hypertensive rats. J Hypertens 2005; 23:971–8
42. Huynh NN, Harris EE, Chin-Dusting JF, Andrews KL: The vascular effects of different arginase inhibitors in rat isolated aorta and mesenteric arteries. Br J Pharmacol 2009; 156:84–93
43. Elms S, Chen F, Wang Y, Qian J, Askari B, Yu Y, Pandey D, Iddings J, Caldwell RB, Fulton DJ: Insights into the arginine paradox: Evidence against the importance of subcellular location of arginase and eNOS. Am J Physiol Heart Circ Physiol 2013; 305:H651–66
44. Rees DD, Palmer RM, Hodson HF, Moncada S: A specific inhibitor of nitric oxide formation from L-arginine attenuates endothelium-dependent relaxation. Br J Pharmacol 1989; 96:418–24
45. Morris SM Jr: Enzymes of arginine metabolism. J Nutr 2004; 134(10 Suppl):2743S–2747S; discussion 2765S–2767S
46. El-Bassossy HM, El-Fawal R, Fahmy A: Arginase inhibition alleviates hypertension associated with diabetes: Effect on endothelial dependent relaxation and NO production. Vascul Pharmacol 2012; 57:194–200
47. Wagner L, Klein JD, Sands JM, Baylis C: Urea transporters are distributed in endothelial cells and mediate inhibition of L-arginine transport. Am J Physiol Renal Physiol 2002; 283:F578–82
48. Hernández G, Tapia P, Alegría L, Soto D, Luengo C, Gomez J, Jarufe N, Achurra P, Rebolledo R, Bruhn A, Castro R, Kattan E, Ospina-Tascón G, Bakker J: Effects of dexmedetomidine and esmolol on systemic hemodynamics and exogenous lactate clearance in early experimental septic shock. Crit Care 2016; 20:234
49. Vanhoutte PM: Regenerated Endothelium and Its Senescent Response to Aggregating Platelets. Circ J 2016; 80:783–90
50. Ebert TJ, Hall JE, Barney JA, Uhrich TD, Colinco MD: The effects of increasing plasma concentrations of dexmedetomidine in humans. Anesthesiology 2000; 93:382–94