

## Direct Coronary and Cerebral Vascular Responses to Dexmedetomidine

### Significance of Endogenous Nitric Oxide Synthesis

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Dexmedetomidine activates  $\alpha_2$ -adrenergic receptors in the central nervous system and in the peripheral vasculature. *In vivo* dexmedetomidine has been found to cause alterations in coronary and cerebral blood flows and arterial pressure by stimulation of vascular smooth muscle  $\alpha_2$  receptors. The direct vasoconstrictor effects of  $\alpha_2$ -adrenergic agonists may be opposed by release of endothelium-derived relaxing factor believed to be nitric oxide. A functional endothelium was demonstrated recently in canine coronary collateral vessels. The objective of the current study was to assess the direct effect of dexmedetomidine on isolated canine proximal and distal coronary arteries, coronary collateral vessels, and middle cerebral arteries. Responses were measured in tissue baths in the presence of indomethacin  $10^{-5}$  M and in the absence and presence of  $N^G$  nitro-L-arginine methyl ester (L-NAME), an inhibitor of vascular nitric oxide synthesis. Dexmedetomidine ( $3 \times 10^{-8}$  to  $3 \times 10^{-5.9}$  M) caused constriction (3.9, 5.5, 72.8, and 2.3% for proximal and distal coronary arteries, middle cerebral arteries, and coronary collateral vessels, respectively, expressed as a percentage of KCl-induced contraction) in all vessels. This constriction was enhanced by the presence of L-NAME in all vessels except cerebral arteries. The selective  $\alpha_2$ -adrenergic antagonist atipamezole ( $10^{-4}$  M) abolished the response to low but not high concentrations of dexmedetomidine in middle cerebral arteries, proximal coronary arteries, and coronary collateral vessels. The response to high concentrations of dexmedetomidine in distal coronary arteries also was abolished by atipamezole. In all other vessels studied, atipamezole only partially antagonized the vasoconstrictor effect of high doses of dexmedetomidine, possibly indicating different mechanisms of action at these concentrations. These results suggest that dexmedetomidine possesses direct vasoconstrictor effects in a variety of isolated vessels. These actions are modulated by vascular nitric oxide synthesis in coronary arteries and coronary collateral vessels but not in middle cerebral arteries. (Key words:  $\alpha_2$  adrenergic receptor agonists: dexmedetomidine.  $\alpha_2$  Adrenergic receptor antagonists: atipamezole. Arteries, coronary:

cerebral; collateral. Endothelium-derived relaxing factor. Receptors, adrenergic:  $\alpha_2$ .)

$\alpha_2$ -ADRENERGIC AGONISTS, including clonidine, possess useful properties as adjuncts for general anesthesia.<sup>1</sup> The  $\alpha_2$ -adrenergic agonist dexmedetomidine<sup>2</sup> has a greater selectivity for the  $\alpha_2$ -adrenergic receptor than does clonidine ( $\alpha_2$  to  $\alpha_1$  ratio 1620:1 vs. 200:1 for clonidine). Dexmedetomidine produces a hypnotic effect and reduces the requirements for inhalational and intravenous (iv) anesthetics.<sup>3-9</sup> These effects may be mediated through specific  $\alpha_2$ -adrenergic receptors within the central nervous system.<sup>10</sup> Traditionally,  $\alpha_2$ -adrenergic receptors were classified as those  $\alpha_2$ -adrenergic receptors situated presynaptically, contrasting with  $\alpha_1$ -receptors, which were located postsynaptically. Peripheral postsynaptic vascular  $\alpha_2$ -adrenergic receptors also exist,<sup>11,12</sup> and alterations in systemic and regional hemodynamics are associated with the use of dexmedetomidine.<sup>13-16</sup> Intravenous administration of dexmedetomidine may result in an abrupt rise in systemic arterial pressure.<sup>15</sup> In anesthetized dogs, coronary blood flow and coronary vascular resistance decreased following iv administration of dexmedetomidine.<sup>17</sup> In contrast, dexmedetomidine produced little change in coronary blood flow in chronically instrumented conscious dogs.<sup>15</sup> Cerebral blood flow also may be altered by dexmedetomidine. In anesthetized dogs, dexmedetomidine decreased cerebral blood flow with little or no effect on the cerebral metabolic rate for oxygen.<sup>13,14</sup> This reduction in blood flow may be mediated by postsynaptic  $\alpha_2$ -adrenergic receptors located within the cerebral vasculature.

Gradual occlusion of an epicardial coronary artery leads to development of collateral vessels supplying the jeopardized area of myocardium.<sup>18</sup> These vessels possess distinct functional characteristics in comparison with normal coronary arteries of similar size, and stimulation of  $\alpha$ -adrenergic receptors may constrict these vessels.<sup>19-24</sup> Recent work demonstrated the presence of endothelium-dependent vasodilation in coronary collateral vessels.<sup>25</sup> The constrictor response to stimulation of  $\alpha_2$ -adrenergic receptors of vascular smooth muscle may be modified by activation of similar receptors on adjacent endothelial cells and subsequent release of endothelium-derived relaxing

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TABLE 1. Number and Sizes of Vessels in Each Group

	Proximal	Distal	Collateral	Cerebral
Number of vessels (number of dogs)	30 (8)	28 (9)	26 (8)	32 (6)
External diameter ( $\mu\text{m}$ ; mean $\pm$ SEM)	1,862 $\pm$ 36	691 $\pm$ 96	820 $\pm$ 63	798 $\pm$ 32

factor (EDRF) believed to be nitric oxide.<sup>26</sup> The occurrence of this phenomenon in the coronary collateral circulation has not been investigated.

The purpose of the present investigation was to examine the direct effects of the  $\alpha_2$ -adrenergic agonist dexmedetomidine on vascular tone in isolated coronary, coronary collateral, and cerebral arteries. Atipamezole is a selective  $\alpha_2$ -adrenergic receptor blocker that antagonizes both central and peripheral effects of  $\alpha_2$ -adrenergic agonists. Since dexmedetomidine may act *via* mechanisms independent of  $\alpha_2$ -adrenergic receptor stimulation, vessels were studied in the presence and absence of atipamezole. Because the action of  $\alpha_2$  agonists may be modulated by simultaneous release of EDRF,<sup>26-28</sup> the vessels also were studied in the presence and absence of an inhibitor of vascular nitric oxide synthase.

### Methods

All experimental procedures and protocols used in this investigation were reviewed and approved by the institutional Animal Care Committee. Furthermore, all experiments conformed to the *Guiding Principles in the Care and Use of Animals* of the American Physiologic Society and were in accordance with the *Guide for the Care and Use of Laboratory Animals*.#

Adult mongrel dogs of either sex were anesthetized with sodium pentobarbital (25 mg/kg, iv), and the heart and brain were rapidly removed. Middle cerebral, proximal left anterior descending, and first and second order branches of the left anterior descending coronary arteries were identified and immediately dissected free, and segments of each vessel were placed in physiologic saline solution (PSS) of the following millimolar composition: NaCl, 119; KCl, 4.7; MgSO<sub>4</sub>, 1.17; CaCl<sub>2</sub>, 1.6; NaHCO<sub>3</sub>, 27.8; NaH<sub>2</sub>PO<sub>4</sub>, 1.18; EDTA, 0.026; glucose, 5.5; and HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid], 5.8. The vessels were cleaned of surrounding fat and connective and other nonvascular tissue, using a dissecting microscope with special care being taken to avoid injury to the luminal surface, and divided into rings of 0.4–0.5 mm diameter. The rings were mounted on tungsten triangles [the lowermost of which was fixed, while the other was attached to a force displacement transducer

(Grass Model FT103, Quincy, MA)] and suspended in jacketed, temperature-controlled (37° C) tissue baths containing PSS aerated with 93.5% O<sub>2</sub> and 6.5% CO<sub>2</sub>. PSS gas tensions were measured every 30 min, and pH and pCO<sub>2</sub> were held constant at 7.38–7.42 and 35 mmHg, respectively. Coronary vascular rings were divided into two groups consisting of small (250–750- $\mu\text{m}$  diameter) and large (1,300–2,500- $\mu\text{m}$  diameter) vessels (table 1). Each ring was gradually stretched to a predetermined optimal resting tension and allowed to equilibrate for at least 2 h. Optimal resting tensions were calculated by measuring the contractile response to 40 mM KCl. The tensions used were: proximal coronary arteries, 1,500 mg; distal coronary arteries, 750 mg; coronary collaterals, 850 mg; and middle cerebral arteries, 750 mg. The transducer signal was processed with an analog-to-digital converter (MacLab<sup>TM</sup>/8, AD Instruments, NSW, Australia) and recorded for later analysis on a microcomputer (Macintosh IIsi, Apple, Cupertino, CA) using dedicated software (Chart v 3.2.2, AD Instruments).

The functional integrity of each ring was assessed by measuring the contractile response evoked by increasing the potassium concentration in the bath to 40 mM. KCl exposure was followed by a washout, and the process was repeated at 30-min intervals until a stable response was obtained. The functional integrity of the vascular endothelium was assessed by determination of the relaxant response to the endothelium-dependent vasodilators, acetylcholine ( $5 \times 10^{-6}$  M) in coronary arteries and the calcium ionophore A23187 ( $10^{-7}$  M) in middle cerebral arteries.

In a separate group of dogs (n = 8), anesthesia was induced with iv sodium thiamylal (10 mg/kg), and after tracheal intubation, anesthesia was maintained with halothane (1.5–2.0% in 100% O<sub>2</sub>). A thoracotomy in the left fifth intercostal space was performed. The lungs were retracted, and the heart was supported in a pericardial cradle. A small segment of the proximal left anterior descending coronary artery was isolated. A hygroscopic Ameroid constrictor (Research Instruments and Manufacturing, Corvallis, OR) was fitted around the vessel to produce a slowly progressive vascular constriction leading to total occlusion over 2–5 weeks. The pericardium was loosely approximated, chest wall closed in layers, and pneumothorax evacuated. All dogs were housed for a minimum of 12 weeks to allow sufficient development of

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collateral vessels. Previous experiments from this laboratory and others have shown that after this period, the levels of blood flow distal to the total coronary artery occlusion are similar to those present in tissue perfused by a normal coronary artery.<sup>29,30</sup>

Dogs with previously implanted Ameroid constrictors were then anesthetized as described above, their trachea intubated, and their lungs ventilated. After thoracotomy was performed, the heart was suspended in a pericardial cradle. Any adherent pericardium was carefully stripped from the epicardial surface. Collateral vessels were easily identified on the epicardial surface of the left ventricle as tortuous, thin, white-to-lucent vessels extending from diagonal branches of the left anterior descending to the marginal branch of the left circumflex coronary artery and from the distal left anterior descending to the posterior descending branch of the left circumflex coronary artery. These vessels ranged from 500 to 1,000  $\mu\text{M}$  in diameter. The mid portion of the collaterals was identified, immediately dissected free, and placed in PSS. Control vessels were obtained from diagonal branches of the left anterior descending coronary artery proximal to the Ameroid constrictor and similarly placed in PSS. Control vessels and collaterals were cleaned, divided into rings, and mounted in tissue baths as described above for cerebral and other coronary arteries. Following repeated exposure to KCl (40 mM), the presence of a functional endothelium was confirmed by observing the relaxant response to the endothelium-dependent vasodilator, acetylcholine.

### EXPERIMENTAL PROTOCOL

Two to three interventions were performed on each vascular ring. Proximal and distal coronary vessels were studied simultaneously. Cerebral vessels were studied separately. Coronary collateral vessels were studied alongside control coronary arteries of a matched size. All experiments were carried out in the presence of indomethacin ( $5 \times 10^{-6}$  M) and propranolol ( $10^{-6}$  M) to eliminate the possible influences of  $\beta$ -adrenergic receptors and vasoactive prostanoids.

#### *Coronary Vessels and Cerebral Vessels*

Following preparation and equilibration, coronary and cerebral arteries were incubated for 20 min with L-NAME ( $10^{-4}$  M) or an equal volume of saline solution. Compared with other inhibitors of nitric oxide synthase, L-NAME demonstrates greater potency both *in vivo* and *in vitro*. Increasing concentrations of dexmedetomidine ( $3 \times 10^{-8}$  to  $3 \times 10^{-3.9}$  M) were then added to each bath to determine cumulative dose-response relationships. Following a further washout, the vessels were again caused to contract with KCl (40 mM), and endothelial function again

assessed using acetylcholine or calcium ionophore. Vasoconstrictor responses to dexmedetomidine also were assessed in the presence and absence of the selective  $\alpha_2$ -adrenergic antagonist atipamezole ( $10^{-4}$  M) in a separate subgroup of vessels previously exposed to L-NAME.

#### *Coronary Collateral Vessels*

After determination of dose-response relationships for dexmedetomidine as described for cerebral and coronary arteries, further experiments were conducted in coronary collateral vessels in the absence and presence of L-NAME ( $10^{-4}$  M). Increasing concentrations of arginine vasopressin ( $10^{-10}$ – $10^{-6}$  M) were added to each tissue bath to obtain cumulative dose-response relationships for comparison. Coronary collateral vessels have been shown previously to possess enhanced sensitivity to vasopressin as compared with control arteries of similar caliber.<sup>22</sup>

Solutions of all drugs were prepared on the day of each experiment. Acetylcholine, arginine vasopressin, calcium ionophore (A23187), and L-NAME were obtained from Sigma Chemical (St. Louis, MO). Dexmedetomidine was a gift of the Research Center of Farnos Group Ltd. (Turku, Finland). All drugs were diluted in distilled water with the exception of calcium ionophore, which was diluted in dimethylsulfoxide.

The contractile responses of all vessels were expressed as a percentage of the KCl (40 mM) reference contraction in each preparation. Responses to each dose of each drug were compared by analysis of variance with repeated measures. If significant differences were found, then comparisons were made using a Duncan's *t* test. The average response of all vessel rings obtained from each dog was calculated and reported as an "n" of 1. Data were expressed as mean  $\pm$  SEM and a *P* value of less than .05 was considered statistically significant.

### Results

Twenty-three dogs were studied (proximal and distal coronary arteries, 9; coronary collateral vessels, 8; cerebral arteries, 6). Vessels previously exposed to L-NAME demonstrated no relaxant response to endothelium-dependent vasodilators when tested at the end of the experiment. Vessels were exposed to a maximal concentration of  $10^{-3.9}$  M dexmedetomidine to avoid  $\alpha_1$ -adrenergic effects. A sigmoid dose-response curve was obtained for middle cerebral arteries. Proximal and distal coronary arteries were exposed to a maximal dexmedetomidine concentration of  $10^{-4.3}$  M.

Proximal coronary arteries demonstrated a small but dose-related constriction to dexmedetomidine ( $3.9 \pm 1.0\%$  of the maximal response of KCl-induced constriction). Exposure to KCl (40 mM) resulted in a contractile response of 3130 mg and 3472 mg in the absence and

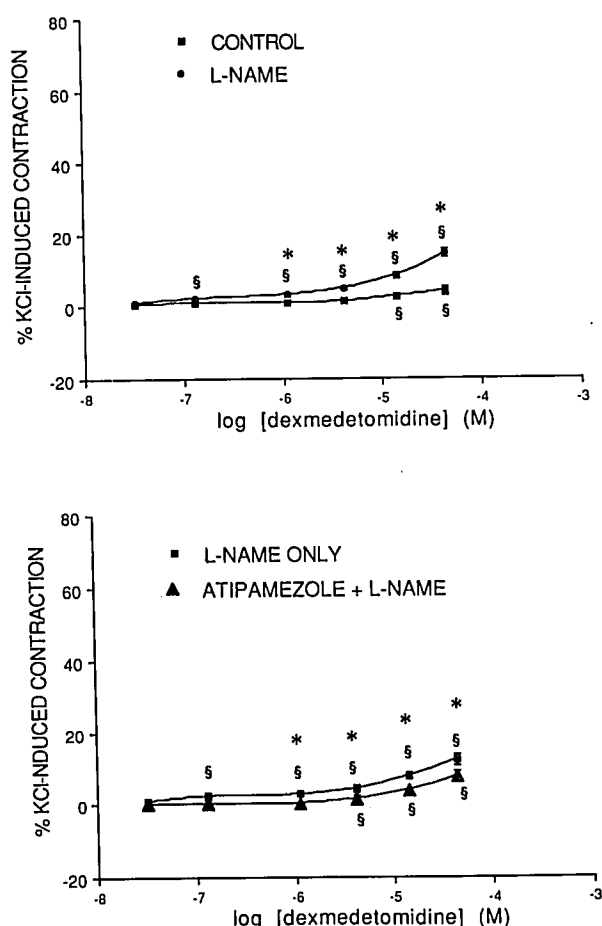


FIG. 1. Isometric tension response to cumulative concentrations of dexmedetomidine in proximal coronary arteries. *Top*: In the absence and presence of N<sup>G</sup>-nitro-L-arginine methyl ester (L-NAME; 10<sup>-4</sup> M). The presence of L-NAME is associated with a significantly enhanced contractile response to dexmedetomidine. *Bottom*: Exposed to L-NAME (10<sup>-4</sup> M) in the absence and presence of the selective  $\alpha_2$ -adrenergic antagonist, atipamezole (10<sup>-4</sup> M). \*Significant ( $P < .05$ ) difference between vessels in each group. §Significant ( $P < .05$ ) contraction compared with tension before intervention.

presence, respectively, of L-NAME. Exposure to L-NAME was associated with a significantly ( $P < .05$ ) enhanced response to dexmedetomidine ( $14.4 \pm 1.4\%$  of the maximal response of KCl-induced constriction; fig. 1, *top*). The dexmedetomidine-induced vasoconstrictor response of proximal coronary arteries exposed to L-NAME was significantly attenuated by the  $\alpha_2$  antagonist atipamezole ( $7.6 \pm 2.1\%$  of the maximal response to KCl-induced constriction; fig. 1, *bottom*).

Distal coronary arteries also were constricted by dexmedetomidine ( $5.5 \pm 1.1\%$  of the maximal response of KCl-induced constriction) in a dose-dependent manner. Exposure to KCl (40 mM) resulted in a contractile response of 1,410 mg and 1,566 mg in the absence and presence, respectively, of L-NAME. Previous exposure to L-NAME produced a significantly ( $P < .05$ ) greater response to

dexmedetomidine ( $11.37 \pm 2.5\%$  of the maximal response of KCl-induced constriction; fig. 2, *top*). Distal coronary arteries exposed to L-NAME and atipamezole showed no significant contractile response to dexmedetomidine (fig. 2, *bottom*).

Middle cerebral arteries demonstrated a dose-related constriction to dexmedetomidine ( $72.8 \pm 5.7\%$  of the maximal response of KCl-induced constriction). Exposure to KCl (40 mM) resulted in a contractile response of 690 mg and 760 mg in the absence and presence, respectively, of L-NAME. In contrast to coronary arteries, previous exposure to L-NAME did not produce a significant alteration in response to dexmedetomidine ( $63.9 \pm 5.7\%$  of the maximal response of KCl-induced constriction; fig. 3, *top*). Atipamezole attenuated the constrictor response of dexmedetomidine in middle cerebral arteries previously exposed to L-NAME ( $19.3 \pm 1.8\%$  of the maximal response of KCl-induced constriction; fig. 3, *bottom*).

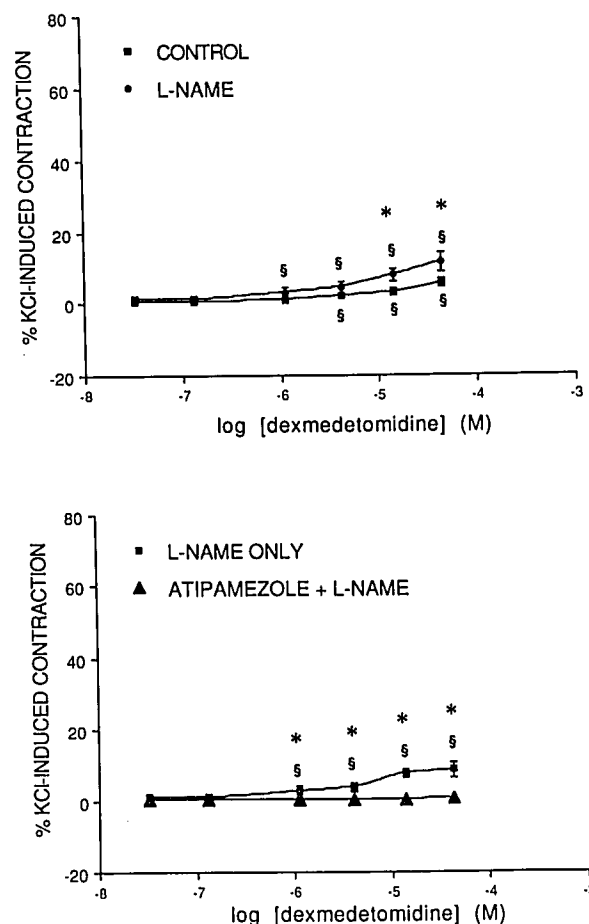


FIG. 2. Isometric tension response to cumulative concentrations of dexmedetomidine in distal coronary arteries. *Top*: In the absence and presence of N<sup>G</sup>-nitro-L-arginine methyl ester (L-NAME, 10<sup>-4</sup> M). *Bottom*: Exposed to L-NAME (10<sup>-4</sup> M) in the absence and presence of the selective  $\alpha_2$ -adrenergic antagonist, atipamezole (10<sup>-4</sup> M). \*Significant ( $P < .05$ ) difference between vessels in each group. §Significant ( $P < .05$ ) contraction compared with tension before intervention.

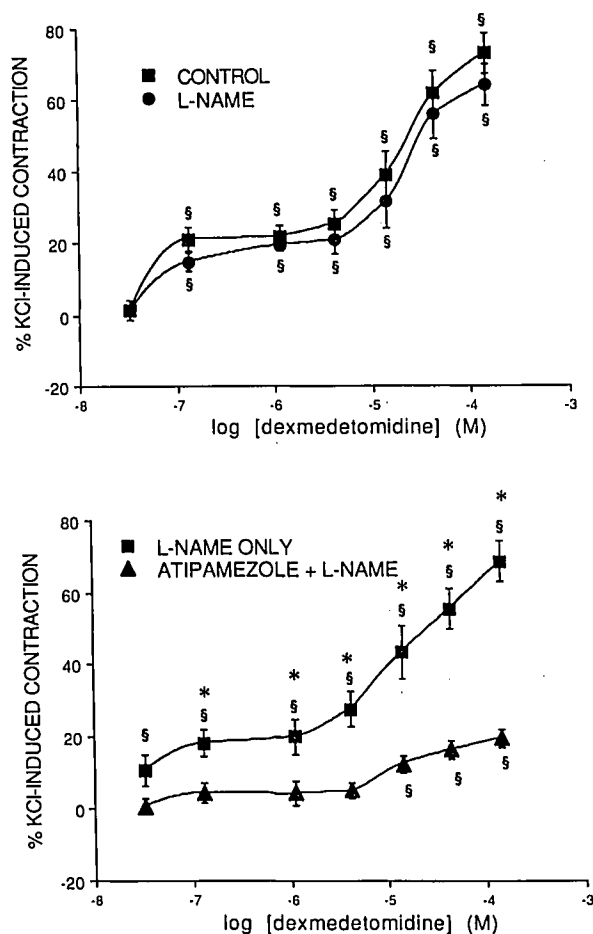


FIG. 3. Isometric tension response to cumulative concentrations of dexmedetomidine in middle cerebral arteries. *Top*: In the absence and presence of  $N^G$ -nitro-L-arginine methyl ester (L-NAME,  $10^{-4}$  M). The respective  $EC_{50}$  values in the absence and presence of L-NAME were  $-4.95 \pm 0.07$  and  $-4.81 \pm 0.08$  (mean  $\pm$  SEM,  $P > .05$ ), respectively. *Bottom*: Exposed to L-NAME ( $10^{-4}$  M) in the absence and presence of the selective  $\alpha_2$ -adrenergic antagonist, atipamezole ( $10^{-4}$  M). \*Significant ( $P < .05$ ) difference between vessels in each group. §Significant ( $P < .05$ ) contraction compared with tension before intervention.

Coronary collateral vessels demonstrated a small constriction in response to dexmedetomidine ( $2.3 \pm 1.27\%$  of the maximal response of KCl-induced constriction at a  $10^{-3.8}$ -M concentration of dexmedetomidine). Exposure to KCl (40 mM) resulted in a contractile response of 816 mg and 893 mg in the absence and presence, respectively, of L-NAME. Pretreatment with L-NAME was associated with an enhanced response to dexmedetomidine ( $12.1 \pm 2.8\%$  of the maximal response of KCl-induced constriction at a  $10^{-3.8}$ -M concentration of dexmedetomidine; fig. 4, *top*). Atipamezole attenuated the constriction response of dexmedetomidine in coronary collaterals exposed to L-NAME ( $2.6 \pm 0.5\%$  of the maximal response of KCl-induced constriction; fig. 4, *middle*). Normal coronary arteries of similar size from dogs with chronic coronary occlusion also constricted following exposure to dexme-

detomidine ( $5.12 \pm 2.5\%$  of the maximum response of KCl-induced constriction). Furthermore, exposure to L-NAME was associated with a significantly enhanced re-

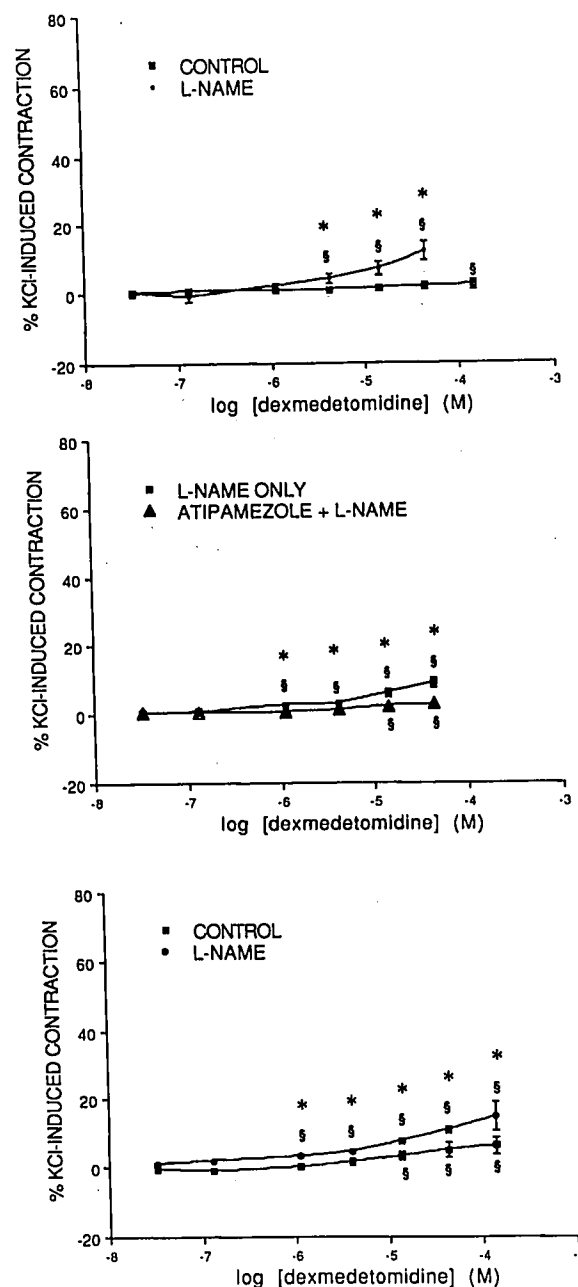


FIG. 4. Isometric tension responses to cumulative concentrations of dexmedetomidine in coronary collateral vessels. *Top*: In the absence and presence of  $N^G$ -nitro-L-arginine methyl ester (L-NAME;  $10^{-4}$  M). *Center*: Exposed to L-NAME ( $10^{-4}$  M) in the absence and presence of the selective  $\alpha_2$ -adrenergic receptor blocker, atipamezole ( $10^{-4}$  M). *Bottom*: Response to dexmedetomidine in the absence and presence of L-NAME ( $10^{-4}$  M) in control coronary arteries of similar dimensions obtained from the same hearts as coronary collateral vessels. \*Significant ( $P < .05$ ) difference between vessels in each group. §Significant ( $P < .05$ ) contraction compared with tension before intervention.

sponse to dexmedetomidine in these vessels ( $14.6 \pm 4.3\%$  of the maximum response of KCl-induced constriction; fig. 4, *bottom*).

For comparison, coronary collateral vessels were constricted by vasopressin ( $80.56 \pm 5.9\%$  of the maximum response of KCl-induced constriction). Pretreatment of collaterals with L-NAME significantly enhanced the constrictor response to vasopressin ( $111.8 \pm 7.0\%$  of the maximum response of KCl-induced constriction; fig. 5, *top*). Control arteries of similar size taken from the same hearts also constricted in response to vasopressin ( $13.05 \pm 4.8\%$  of the maximum response of KCl-induced constriction; fig. 5, *bottom*), but this response was significantly

( $P < .05$ ) less than that obtained using coronary collateral vessels. In contrast to collaterals, prior exposure of the control coronary arteries (taken from the same hearts) to L-NAME was not associated with an enhanced response to vasopressin ( $11.9 \pm 3.0\%$  of the maximum response of KCl-induced constriction).

## Discussion

The results of the present investigation demonstrate: 1) the vasoconstrictor effect of the  $\alpha_2$ -adrenergic agonist dexmedetomidine on isolated proximal and distal coronary arteries, middle cerebral arteries, and coronary collateral vessels; 2) the antagonism of dexmedetomidine-induced vasoconstriction in these vessels by the selective  $\alpha_2$ -blocking agent atipamezole; 3) the augmentation of the constrictor effect of dexmedetomidine by inhibition of vascular nitric oxide synthesis in coronary but not cerebral vessels; and 4) the augmentation of the effect of vasopressin by inhibition of vascular nitric oxide synthesis in coronary collateral vessels.

$\alpha_2$ -Adrenergic agonists possess properties that may serve a useful adjunctive role in anesthetic practice.<sup>1</sup> These include improved hemodynamic stability, sedation, and a reduction in anesthetic and analgesic requirements.<sup>3-9</sup> Stimulation of presynaptic  $\alpha_2$ -adrenergic receptors in the central nervous system has been shown to reduce noradrenaline release from adrenergic neurons. This offers a mechanism for the anesthetic effect of dexmedetomidine. However, the majority of  $\alpha_2$ -adrenergic receptors may be situated postsynaptically.<sup>31</sup> Drugs, such as clonidine, that activate central  $\alpha_2$ -adrenergic receptors also demonstrate useful antihypertensive effects. In contrast, activation of peripheral  $\alpha_2$ -adrenergic receptors may result in vasoconstriction and increased systemic arterial pressure.<sup>32</sup>

Peripheral  $\alpha_2$ -adrenergic receptors may be situated postsynaptically, and the activation of these receptors is followed by an influx of extracellular calcium and contraction of vascular smooth muscle.<sup>11,12,33</sup> The isolated vessel model employed in the current series of experiments is suitable for the study of postsynaptic receptor effects. In the conscious, chronically instrumented dog, iv administration of dexmedetomidine causes an initial increase in systemic arterial blood pressure.<sup>15</sup> This effect is consistent with the contractile response observed in isolated arteries of several types observed in the present investigation.

While coronary blood flow is predominantly subject to metabolic rather than neurogenic influences, both  $\alpha_1$  and  $\alpha_2$  adrenoreceptors are present in coronary epicardial vessels and may reduce coronary artery diameter and perfusion under certain conditions.<sup>34</sup> A differential distribution of  $\alpha_1$ - and  $\alpha_2$ -adrenergic receptors between proximal and distal coronary arteries has been suggested

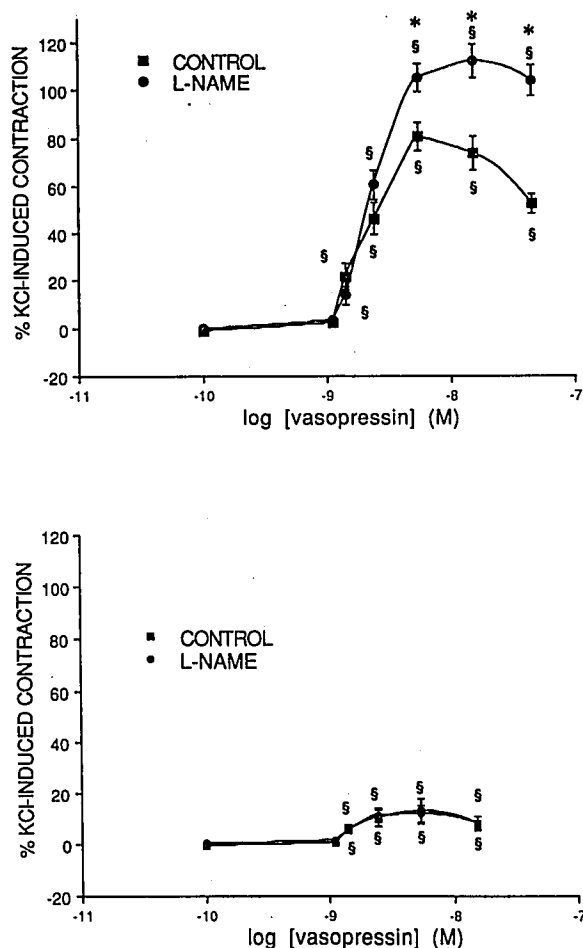


FIG. 5. Isometric tension response to cumulative concentrations of vasopressin in coronary collateral vessels (*top*) and control arteries of similar diameter taken from the same hearts (*bottom*) in the absence and presence of N<sup>G</sup>-nitro-L-arginine methyl ester (L-NAME;  $10^{-4}$  M). The respective EC<sub>50</sub> (log molar concentrations of vasopressin, mean  $\pm$  SEM) values for collaterals in the presence and absence of L-NAME were  $-8.79 \pm 0.11$  and  $-8.68 \pm 0.09$ , respectively ( $P > .05$ ). In control arteries EC<sub>50</sub> values in the presence and absence of L-NAME were  $-8.88 \pm 0.03$  and  $-8.86 \pm 0.02$ , respectively ( $P > .05$ ). \*Significant ( $P < .05$ ) difference between vessels with and without L-NAME. §Significant ( $P < .05$ ) contraction compared with tension before intervention.

previously.<sup>35</sup> Heusch *et al.* have described a predominance of  $\alpha_2$ -adrenergic effects in distal resistance vessels while  $\alpha_1$ -adrenergic effects were found to be present primarily in large epicardial arteries.<sup>35</sup> The  $\alpha_1$ -adrenergic receptor agonist methoxamine has been shown to produce constriction of large epicardial coronary arteries and increase coronary vascular resistance *in vivo*.<sup>34</sup> Using intravital microscopy of the subepicardial microcirculation, Chilian<sup>36</sup> demonstrated  $\alpha_1$ -adrenergic constriction of small arteries (153- $\mu$ M) and arterioles (73- $\mu$ M) and exclusive  $\alpha_2$ -adrenergic constriction of arterioles. In the present study, using an isolated vessel technique, both proximal ( $1851 \pm 44$ - $\mu$ M) and distal ( $470 \pm 32$ - $\mu$ M) epicardial vessels demonstrated contractile responses to dexmedetomidine.

Recently, the capacity of dexmedetomidine (especially at high concentrations) to stimulate  $\alpha_1$ -adrenergic receptors was suggested.<sup>37,38</sup> In the present investigation, despite the presence of the selective  $\alpha_2$ -adrenergic antagonist atipamezole, persistence of a contractile response at high concentrations of dexmedetomidine in proximal coronary arteries was shown. Atipamezole, however, totally blocked the constriction in distal coronary vessels. These results are consistent with stimulation of  $\alpha_1$ -adrenergic receptors by high concentrations of dexmedetomidine in proximal coronary arteries and the absence of this phenomenon in distal arteries. Thus, there may be a relative predominance of  $\alpha_2$ -adrenergic receptors on more distal vessels, as suggested by Heusch *et al.*<sup>35</sup> It has been suggested that  $\alpha_2$  adrenoceptors are the most significant subtype in mediating coronary vasoconstrictor responses to cardiac nerve stimulation.<sup>39</sup> Flacke *et al.*<sup>17</sup> have shown a reduction in coronary blood flow during an increase in myocardial  $O_2$  extraction immediately following administration of dexmedetomidine in anesthetized dogs. By contrast, in conscious dogs, no changes in coronary blood flow occurred.<sup>15</sup> Despite the use of concentrations of dexmedetomidine up to  $10^{-4.3}$  M in the present study, a plateau contractile response was not reached in the coronary vessels studied. This may reflect, as Vanhoutte has suggested, the lack of a large receptor reserve for  $\alpha_2$ -adrenergic receptor agonists on these vessels.<sup>26</sup> The effect of a functional antagonist such as nitric oxide may be quite marked under such circumstances, effectively inhibiting  $\alpha_2$ -adrenergic receptor-mediated contractions.

Evidence exists for the mediation of cerebral vasoconstriction by  $\alpha_2$ -adrenergic receptors. Postsynaptic  $\alpha_2$ -adrenergic receptors are present in the canine middle cerebral artery.<sup>40</sup> Experimental hypovolemic hypotension and concomitant sympathetic nervous system activation caused cerebral vasoconstriction that was prevented by  $\alpha_2$ -adrenergic blockade in the dog.<sup>33</sup> A reduction of cerebral blood flow in humans following administration of clonidine has been described.<sup>41</sup> During isoflurane or halothane anesthesia in the dog, dexmedetomidine caused a reduction in cerebral blood flow with little or no effect

on the global cerebral metabolic rate for oxygen.<sup>13,14</sup> Karlsson *et al.*<sup>14</sup> were unable, however, to determine whether the decline in cerebral blood flow was due to direct cerebral vasoconstriction or an indirect attenuation of the vasodilating effect of halothane. The present investigation has confirmed that the direct vasoconstrictor effect of dexmedetomidine on the canine middle cerebral artery is mediated by postsynaptic  $\alpha_2$  adrenoceptors. The failure of the selective  $\alpha_2$ -adrenergic blocking agent atipamezole to block cerebral artery vasoconstriction at higher concentrations of dexmedetomidine may suggest some stimulation of  $\alpha_1$ -adrenergic receptors in these vessels as well. Vessels distal to the middle cerebral artery were not examined in the current study. However, within the cerebral circulation, the middle cerebral artery contributes substantially to total cerebrovascular resistance.<sup>42</sup>

Under certain conditions, the contractile response to  $\alpha_2$ -adrenergic stimulation may be inhibited by the presence of a functional vascular endothelium and release of EDRF.<sup>26,28</sup> A basal release of EDRF may nonspecifically oppose the effect of stimulation of  $\alpha_2$ -adrenergic receptors on vascular smooth muscle to cause vasoconstriction. Simultaneous activation of  $\alpha_2$ -adrenergic receptors on endothelial cells also may release EDRF and oppose the actions of  $\alpha_2$ -receptor stimulation of vascular smooth muscle. In the present investigation, the vasoconstrictor response of dexmedetomidine was assessed in coronary and cerebral arteries in the presence and absence of the competitive inhibitor of nitric oxide synthesis, L-NAME. Inhibition of nitric oxide synthesis was associated with an exaggerated constrictor response to dexmedetomidine in coronary but not in cerebral arteries. These results suggest that the response to  $\alpha_2$ -adrenergic agonists is not modified by release of EDRF in canine middle cerebral arteries. The magnitude of the contractile response to dexmedetomidine in cerebral arteries was much greater than that of coronary arteries. The greater cerebral vasoconstriction may minimize any modifying effect of basal nitric oxide release. These findings also may be explained by the absence of  $\alpha_2$ -adrenergic receptors on vascular endothelial cells of middle cerebral as opposed to coronary arteries in the dog. Studies using hemoglobin/methylene blue and incorporating dose-response studies at various concentrations of atipamezole and L-NAME, with measurement of cyclic GMP levels, would further assist in outlining the mechanisms involved in the differential vascular responses to dexmedetomidine and the co-release of nitric oxide.

Gradual occlusion of an epicardial coronary artery leads to growth of collateral vessels that supply the potentially ischemic area of myocardium.<sup>18</sup> Marked structural differences exist between coronary collateral vessels and normal arteries of the same size.<sup>21</sup> Angus *et al.*<sup>21</sup> have demonstrated an impaired contractile performance of vascular smooth muscle in coronary collaterals at 24 weeks

of development. These authors concluded that collaterals were compromised flow conduits because of encroachment of the neointima on the vessel lumen. Differences in the responses of coronary collaterals to  $\alpha_2$ -adrenergic agonists and vasopressin have been described also.<sup>22</sup> Furthermore, conflicting results have been reported from investigations of  $\alpha_2$ -adrenergic responses. Transcollateral resistance in an isolated blood-perfused heart preparation and isometric tension of isolated collateral vessels have been described to be unaffected by clonidine.<sup>20</sup> Using a different *in vivo* preparation, the selective  $\alpha_2$  agonist B-HT 920 has been shown to cause an increase in transcollateral resistance.<sup>24</sup> The potential modification of  $\alpha_2$ -adrenergic responses by a functional vascular endothelium was not explored in the preceding studies. However, the presence of functional endothelium capable of releasing EDRF in coronary collateral vessels was demonstrated recently.<sup>25</sup> In the present investigation, dexmedetomidine caused only a small increase in coronary collateral vessel tone, but this action was enhanced markedly in the presence of L-NAME. The vasoconstriction was blocked by the selective  $\alpha_2$ -antagonist atipamezole. These results are consistent with the mediation of constriction in coronary collaterals by  $\alpha_2$ -adrenergic receptors, the modulation of this effect by nitric oxide of vascular origin, and a paucity or absence of  $\alpha_1$ -adrenergic receptors in these vessels.

The peptide hormone vasopressin may play a significant role in the regulation of systemic vascular resistance and regional blood flow with variable effects on regional perfusion.<sup>43</sup> This may be associated with a similar variation in endothelium-dependent responses to vasopressin. Katusic *et al.* reported endothelium-dependent relaxations mediated by  $v_1$  receptors in canine basilar arteries.<sup>44</sup> This effect was less marked in coronary and absent in femoral arteries. In the coronary circulation, vasopressin constricts resistance arteries and causes dilation of proximal coronary arteries *via* a mechanism partially dependent upon vascular endothelium.<sup>45,46</sup> Harrison and Peters<sup>20,23</sup> have demonstrated an increased responsiveness to vasopressin in mature collateral vessels when compared with normal arteries of similar caliber. Vasoconstriction of collateral vessels has been shown to occur at relatively low concentrations of vasopressin and may have important clinical implications.<sup>20,23</sup> The possible modification of the response to vasopressin by EDRF was explored in the present study because of the recent demonstration of endothelium-dependent vasodilation in canine coronary collateral vessels.<sup>25</sup> The exaggerated response of collateral vessels as compared to normal coronary arteries by vasopressin confirmed the findings of Harrison and Peters.<sup>20,23</sup> The enhancement of this response by inhibition of nitric oxide synthase indicates the capacity of the coronary collateral to modify the constrictor effect of vasopressin through production of EDRF.

The present investigation describes the production by

dexmedetomidine of constriction in the range of 2–20% of the KCl-induced contraction of isolated coronary arteries and coronary collateral vessels. The vasoconstriction was maximized by inhibition of nitric oxide synthesis and the use of high concentrations of dexmedetomidine, which may be associated with stimulation of  $\alpha_1$ -adrenergic receptors. These results suggest that coronary arterial and collateral circulations may be constricted by  $\alpha_2$ -adrenergic agonists. The present findings, however, are based on observations in the isolated vessel, and other non-neurogenic factors are of major importance in the regulation of coronary blood flow. Furthermore, the direct stimulation of  $\alpha_2$  receptors in vascular smooth muscle results in only minimal constriction. By comparison, vasopressin has a profound vasoconstrictor action. Concentrations of dexmedetomidine at receptor sites *in vivo* may be much smaller than that apparent *in vitro*, especially following intramuscular or oral drug administration. Finally, dexmedetomidine *in vivo* may reduce sympathetic nervous system tone to the coronary circulation *via* central mechanisms, and vascular endothelium may provide an endogenous protection against  $\alpha_2$  adrenoceptor-mediated vasoconstriction.

The combination of a volatile halogenated anesthetic and dexmedetomidine in the dog has been shown to be associated with a decrease in the cerebral metabolic rate for oxygen and a neutral effect on cerebral blood flow resulting from opposing effects of the inhalational agent and dexmedetomidine on cerebrovascular tone.<sup>14</sup> However, considerable interspecies variation exists with respect to regulation of vascular tone. While it is known that clonidine may be associated with a reduction in cerebral blood flow in the human,<sup>41</sup> the effects of dexmedetomidine on cerebral blood flow in humans have yet to be studied.

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