Effects of Cocaine on Nitric Oxide Production in Bovine Coronary Artery Endothelial Cells

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ABSTRACT

Cocaine decreases coronary artery endothelial-dependent vasorelaxation. To explore the potential mechanisms, the present study examined the effect of cocaine on nitric oxide release in bovine coronary artery endothelial cells (BCAECs). In the absence of cocaine, basal nitric oxide release from BCAECs continued to accumulate in the medium over the period from 6 to 72 h. Cocaine significantly decreased nitric oxide release at each time point of the study. At 48-h treatment, cocaine (3–30 μM) produced a concentration-dependent decrease in nitric oxide release in BCAECs. In accordance with its inhibition of nitric oxide release, cocaine decreased endothelial nitric-oxide synthase (eNOS) protein levels in BCAECs in a concentration-dependent manner. In addition to the prolonged effect, cocaine pretreatment for 1 h significantly decreased basal and ATP-induced nitric oxide release in BCAECs. Whereas acute cocaine treatment did not affect basal levels of free intracellular calcium concentrations in BCAECs, it significantly decreased the ATP-induced elevation of intracellular calcium and increased its time lag to reach the peak. A quantitative approach by immunofluorescence microscopy revealed that cocaine significantly increased eNOS localized at the cell membrane in BCAECs. Collectively, the results suggest that cocaine inhibits nitric oxide release in BCAECs by decreasing intracellular calcium mobilization, increasing the inactive state of eNOS, and decreasing eNOS protein levels.

The complications of cocaine abuse affect every system of the body. The multifactorial effects of cocaine on the cardiovascular system often contribute to its sympathomimetic action. It has been suggested that the coronary artery is more sensitive to endogenous vasoactive substances after chronic cocaine abuse (Jones and Tackett, 1990). Cocaine abuse has been reported to cause an increase in coronary artery vasoconstriction (Vongpatanasin et al., 1997) and recurrent coronary vasoconstriction (Brogan et al., 1992), a decrease in coronary blood flow (Miao et al., 1996), and an increase in myocardial ischemia and infarction (Pitts et al., 1997). Although acute cocaine use is typically considered as a risk factor for acute cardiac events, chronic use may contribute to the development or rapid progression of coronary artery diseases (Wilson, 1998; He et al., 2000).

Previous studies have suggested that the endothelium-dependent vasorelaxation is impaired in long-term cocaine abusers (Wang et al., 1995; Havranek et al., 1996). In addition, experimental results showed that stripping the endothelial layer from isolated arteries abolished the vasoconstrictive effect of cocaine (Mo et al., 1998). These studies suggest that the vasoconstrictive action of cocaine is mediated at least in part by inhibiting endothelial nitric oxide synthesis. The endothelium plays an important role in modulating coronary vascular tone by releasing nitric oxide, and inhibition of nitric oxide generation from coronary endothelium results in an increase in coronary vascular resistance and attenuation of flow-induced coronary vascular dilation (Chu et al., 1991). Abnormal coronary endothelial function could contribute to atherosclerosis, thrombosis, focal vasospasm, and myocardial ischemia, all of which have been demonstrated in cocaine users with myocardial infarction (Havranek et al., 1996).

Nitric oxide is synthesized in the endothelium by endothelial nitric-oxide synthase (eNOS). In contrast to the other nitric-oxide synthase isoforms, eNOS binds to the particular subcellular fractions. It has been suggested that the eNOS enzyme is tonically inhibited by its protein-protein interaction with caveolin, the resident scaffolding protein in plasmalemmal caveolae (Michel and Feron, 1997). The dynamic equilibrium of eNOS plasmalemmal membrane targeting in

ABBREVIATIONS: eNOS, endothelial nitric-oxide synthase; PBS, phosphate-buffered saline; BCAEC, bovine coronary artery endothelial cell; NOx, combined concentrations of nitrate, nitrite, and nitric oxide; [Ca2+]i, intracellular free Ca2+ concentration.
cultured endothelial cells is exquisitely sensitive to changes in intracellular calcium concentrations. It has been demonstrated that agonist-induced activation of eNOS is mediated by translocation of eNOS from plasmalemmal membrane to intracellular sites close to the nucleus, and the translocation is completely abrogated by chelation of intracellular Ca2+ (Prabhakar et al., 1998).

To our knowledge, there is no study yet that directly examines the effect of cocaine on nitric oxide release in the endothelium. The specific objectives of this study therefore were to determine in bovine coronary artery endothelial cells whether cocaine 1) decreased nitric oxide production, 2) inhibited intracellular Ca2+ mobilization, 3) changed dynamic equilibrium of eNOS distribution between plasmalemmal membrane and intracellular sites close to the nucleus, and 4) decreased eNOS protein levels.

Materials and Methods

Materials. Cocaine, phosphate-buffered saline (PBS), ATP, and anti-actin monoclonal antibody were purchased from Sigma (St. Louis, MO). Vanadium(III) chloride was from Aldrich Chemical Co. (Milwaukee, WI). Fetal bovine serum was purchased from HyClone Laboratories (Logan, UT). Protein assay kit was from Bio-Rad (Hercules, CA). Purified anti-eNOS antibody was from BD Biosciences (Franklin Lakes, NJ). Horseradish peroxidase-conjugated anti-mouse IgG was from Amersham Biosciences, Inc. (Clearbrook, IL). Anti-mouse IgG-fluorescein isothiocyanate isothiocyanate was from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Prestained protein molecular weight standards were from Invitrogen (Carlsbad, CA). Fura-2/acetoxymethyl ester was from Molecular Probes (Eugene, OR).

Cell Culture. Bovine coronary artery endothelial cells (BCECs) were obtained from Cell Applications, Inc. (San Diego, CA). Cells were grown in the complete medium of Dulbecco’s modified Eagle’s medium (Mediatech, Herndon, VA) with 4.5 g/l glucose, 15% fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin and were incubated at 37°C in a humidified incubator with 5% CO2, 95% air. Cells were used for the experiments at the fifth and sixth passages. Cell numbers were determined using a hemacytometer, and cell viability was determined using trypan blue exclusion.

Measurement of Intracellular Free Ca2+ Concentration ([Ca2+]i). [Ca2+]i was measured in single cells as described previously (Prabhakar et al., 1998; Goetz et al., 1999). Briefly, after cocaine treatment for 1 h, cells were fixed in 2% freshly prepared paraformaldehyde for 10 min at room temperature and permeabilized in PBS with 0.1% Triton X-100 and 0.1% bovine serum albumin for 5 min at room temperature. Cells were then incubated in PBS containing 10% bovine calf serum overnight at 4°C, treated with anti-eNOS monoclonal antibody at 1:250 dilution for 60 min at room temperature, washed with PBS three times for 5 min each, and incubated with anti-mouse IgG-fluorescein isothiocyanate antibody at 1:200 dilution for 60 min at room temperature. After washing with PBS three times for 10 min each, the coverslips bearing the cells were mounted on a microscope slide and visualized by fluorescence microscope. Quantitative analysis of eNOS immunofluorescence was performed as described previously (Prabhakar et al., 1998; Goetz et al., 1999). Since the eNOS immunofluorescence at the membrane was usually nonuniform in each individual cell, a cell was assigned as plasmalemmal membrane-bound eNOS-state when even a small patch of eNOS was detected at plasmalemmal membrane (state A). On the other hand, if the eNOS immunofluorescence was visualized only in the cytosol, the cell was assigned as cytosolic eNOS-state (state C). If the eNOS immunofluorescence was visualized in both the plasmalemmal membrane and the cytosol, the cell was assigned as mixed eNOS-state (state B). For each experiment, the pictures of cells taken under fluorescent microscopy in nine randomly selected high power fields were analyzed (approximately 200 cells/coverslips). The percentage of each eNOS state was compared between the control and cocaine-treated samples. Each experiment was conducted at least three times. The proportion of untreated cells scored as eNOS membrane-positive was very consistent under identical experimental conditions, and inter-experimental variability was generally less than 5%.

Western Analysis of eNOS. After the treatment, the endothelial cells were solubilized by sonication in lysis buffer (150 mM NaCl, 50 mM Tris-HCl, 10 mM EDTA, 0.1% Tween 20, 0.1% β-mercaptoethanol, 0.1 mM phenylmethylsulfonyl fluoride, 5 µg/ml leupeptin, and 5 µg/ml aprotinin, pH 7.4). After centrifugation, protein was quantified in the supernatant. Samples with equal protein (20 µg) were loaded on a 7.0% polyacrylamide gel with 0.1% SDS and were separated by electrophoresis at 100 V for 2 h. Proteins were then transferred onto Immobilon P membrane at 30 V for 30 min at room temperature using a semi-dry blotter (Bio-Rad). The Immobilon P membrane was probed by mouse monoclonal antiserum for eNOS (1:750) obtained from BD Transduction Laboratories (Lexington, KY). The secondary antiserum was horseradish peroxidase-conju-
gated goat anti-mouse (1:1000) obtained from Amersham Biosciences, Inc. (Piscataway, NJ). Proteins were visualized with enhanced chemiluminescence reagents (Amersham Biosciences, Inc.), and the blots were exposed to Hyperfilm. Results were quantified by scanning densitometer (model 670; Bio-Rad) and expressed as percentage of the control values.

Data Analysis. Data were presented as the mean ± S.E.M. Statistical analyses were performed by paired Student’s t test as well as one-way analysis of variance followed by Newman-Keuls post hoc tests, respectively. Differences were considered significant at $P < 0.05$.

Results

Effect of Cocaine on Nitric Oxide Release. The effect of cocaine on ATP-induced NO$_x$ release in BCAECs is shown in Fig. 1. Cells were treated with control medium or medium with 100 µM cocaine for 1 h and were then challenged with different concentrations of ATP for 1 h. Nitric oxide (measured as NO$_x$) in the medium was assayed by the chemiluminescence method. As shown in Fig. 1, the cocaine treatment significantly decreased the basal levels of NO$_x$ from $215.6 ± 77.6$ to $20.7 ± 10.5$ ($P < 0.05$). ATP (1–100 µM) produced a concentration-dependent increase in NO$_x$ release in both control and cocaine-treated cells. The pD$_2$ values (5.45 ± 0.66 versus 5.51 ± 0.24; $P > 0.05$) and the ATP-induced maximal net increase of NO$_x$ (232.7 ± 58.1 versus 242.7 ± 20.3; $P > 0.05$) were not significantly different between the two groups. The effect of prolonged cocaine treatment on basal NO$_x$ release in BCAECs is shown in Fig. 2. At 48-h treatment, cocaine (3, 10, and 30 µM) produced a concentration-dependent decrease in basal NO$_x$ release in BCAECs (Fig. 2).

Effect of Cocaine on Intracellular Ca$^{2+}$ Mobilization. [Ca$^{2+}$]$_i$ in BCAECs was examined in single cells loaded with Fura-2. Our previous studies showed that ATP (1–300 µM) induced a concentration-dependent increase in [Ca$^{2+}$]$_i$ in coronary artery endothelial cells (Yang et al., 2000). Figure 3 shows representative single cell fluorescence tracing stimulated with a submaximal concentration (~70% of the maximal effect) of 30 µM ATP. As shown in the figure, ATP induced a rapid rise in [Ca$^{2+}$]$_i$ in BCAECs, which was reflected by an increase in Fura-2 fluorescence intensity at 340 nm$_{ex}$ and a decrease in the intensity at 380 nm$_{em}$. The maximal [Ca$^{2+}$]$_i$ response was reached within 20 s followed by a decline to a steady state above the baseline level (Fig. 3). Unlike ATP, cocaine (30 µM) did not change basal [Ca$^{2+}$]$_i$ in BCAECs (38.47 ± 4.25 versus 40.99 ± 5.11 nM). Figure 4 shows the real-time effect of cocaine on ATP-induced [Ca$^{2+}$]$_i$ response in BCAECs. As shown in Fig. 4, 30 µM ATP caused Fig. 1. Effect of cocaine on ATP-induced nitric oxide release in BCAECs. BCAECs were pretreated in the absence and presence of cocaine (100 µM) for 1 h, followed by stimulation with indicated concentrations of ATP for 1 h. NO$_x$ in the medium was measured by the chemiluminescence method as described under Materials and Methods. B, basal levels. Data are means ± S.E.M. of 12 experiments.

Fig. 2. Concentration-dependent effect of cocaine on nitric oxide release in BCAECs. BCAECs were treated in the absence and presence of cocaine (3, 10, and 30 µM) for 48 h. NO$_x$ in the medium was measured by the chemiluminescence method as described under Materials and Methods. Data are means ± S.E.M. of six experiments. *, $P < 0.05$ versus the control.

Fig. 3. Effect of ATP on single cell [Ca$^{2+}$]$_i$ in BCAECs. Representative fluorescence tracings of Fura-2 loaded single cell in response to 30 µM ATP stimulation. Top, fluorescence intensity recorded at excitation wavelength of 340 nm. Middle, fluorescence intensity recorded at excitation wavelength of 380 nm. Bottom, intracellular Ca$^{2+}$ transient calculated by the ratios of F$_{340}$/F$_{380}$ as described under Materials and Methods.
a [Ca\(^{2+}\)]\(_i\) spike followed by a plateau phase in a single endothelial cell (Fig. 4A). After washing and recovery, the same cell was pretreated with 30 \(\mu\)M cocaine for 15 min and then challenged again with 30 \(\mu\)M ATP (Fig. 4B), and the [Ca\(^{2+}\)]\(_i\) peak induced by ATP was significantly reduced. The response to ATP was completely recovered after the removal of cocaine (Fig. 4C). Quantitative analysis of the data revealed that the [Ca\(^{2+}\)]\(_i\) peaks induced by 30 \(\mu\)M ATP in the absence and presence of cocaine were 170.3 ± 45.9 versus 92.8 ± 31.3 nM, respectively \((P < 0.05; \text{paired } t \text{ test})\) (Fig. 5A). In addition to its effect on the peak [Ca\(^{2+}\)]\(_i\) response induced by ATP, cocaine significantly increased the time lag of ATP-stimulated [Ca\(^{2+}\)]\(_i\) response in BCAECs. As shown in Fig. 5B, the time required between ATP administration and the peak [Ca\(^{2+}\)]\(_i\) response in the absence and presence of cocaine was 11.3 ± 2.0 and 25.1 ± 4.5 s, respectively \((P < 0.05; \text{paired } t \text{ test})\).

**Effect of Cocaine on eNOS Translocation.** eNOS is a Ca\(^{2+}\)-dependent enzyme and is subject to a complex pattern of intracellular regulation. Its activation includes the reversible translocation from plasmalemmal membrane to intracellular sites close to the nucleus. In resting cells, eNOS is tonically inhibited by its binding to caveolin at the plasmalemmal caveolae. The dynamic distribution of eNOS between plasmalemmal membrane and the intracellular sites is exquisitely sensitive to changes in intracellular calcium concentrations. To study the effect of cocaine on eNOS translocation, BCAECs cultured for 24 h in the serum-free medium were treated with cocaine (100 \(\mu\)M) for 1 h. Figure 6 (top) shows the typical photomicrographs of BCAECs processed for immunolabeling of eNOS. The cellular distribution of eNOS immunoreactivity exhibited considerable heterogeneity. As shown in Fig. 6, three distribution states of eNOS were noted.
in BCAECs: plasmalemmal membrane-bound eNOS-state (state A), cytosolic eNOS-state (state C), and mixed eNOS-state (state B). In the absence of cocaine, about 70% of cells showed eNOS in the cytosolic fraction (state C). Cocaine treatment for 1 h significantly increased membrane-bound eNOS (state A) but decreased eNOS in state B and C (Fig. 6, bottom).

**Effect of Cocaine on eNOS Protein Levels.** Figure 7 shows the effect of prolonged cocaine treatment on eNOS protein levels in BCAEC. The representative Western immunoblot showed that the monoclonal antibody for eNOS detected a single band at the expected size of 135 kDa (Fig. 7, top). Cocaine (10, 30, and 100 μM) treatment for 48 h produced a concentration-dependent decrease in eNOS protein levels in BCAECs. Quantitative densitometry for four independent experiments revealed that cocaine produced more than 70% decrease in eNOS protein levels in BCAECs (Fig. 7, bottom).

**Discussion**

The present study investigated the inhibitory effects of cocaine on nitric oxide production and the potential underlying mechanisms in cultured bovine coronary artery endothelial cells. The major findings are as follows: 1) both acute and prolonged cocaine treatments decrease nitric oxide release, 2) acute cocaine treatment inhibits intracellular Ca\(^{2+}\) mobilization, 3) cocaine drives eNOS translocation to an inactive plasmalemmal membrane binding state, and 4) prolonged cocaine treatment decreases eNOS protein levels.

The present study demonstrated for the first time that cocaine indeed inhibited nitric oxide release in coronary artery endothelial cells. The range of cocaine concentrations (3–100 μM) used in the present study is relevant to human exposure levels. Because serum levels of cocaine in active drug abusers are often >100 μM and the repeated use of cocaine produce dose-related accumulation in serum cocaine concentrations (Nassogne et al., 1997; Jufer et al., 1998), the pathophysiological relevance of the present finding is fully warranted. The finding that the basal release of nitric oxide was concentration dependently decreased with the exposure to cocaine for 48 h suggests that long-term cocaine action may interfere with the intrinsic endothelial nitric oxide synthesis system. Similar findings were obtained in human coronary artery endothelial cells exposed to erythropoietin (Wang and Vaziri, 1999). Further experiments revealed that the fall in nitric oxide production was accompanied by a parallel concentration-dependent reduction in eNOS protein levels in BCAECs after exposure to cocaine for 48 h. Because eNOS is the enzyme responsible for nitric oxide production in endothelial cells, the decreased expression of the protein may account for the corresponding decrease of basal nitric oxide release in prolonged cocaine treatment in these cells. Previous studies have suggested that decreased eNOS expression is associated with endothelial dysfunction in a variety of diseases (Agnoletti et al., 1999; Toporsian et al., 2000). Our current results provide a partial mechanistic explanation for coronary artery vasospasm and recurrent coronary vasoconstriction and ischemia in chronic cocaine abusers because the down-regulation of eNOS may form the pathological basis of the progression of coronary artery diseases. Moreover, synthesis of both endothelin-1 and thromboxane A\(_2\) has been reported to be under the negative regulatory influence of nitric oxide (Goligorsky et al., 1994; Wade and Fitzpatrick, 1997). Accordingly, cocaine-induced inhibition of eNOS expression and decrease in nitric oxide release may play an additional role in the alteration in endothelial function through the removal of an inhibitor of vasoconstrictors.

It is now well established that the endothelium-dependent vasodilation produced by vasoactive agents such as ATP is through the increase of [Ca\(^{2+}\)]\(_i\) and stimulation of the calcium-dependent eNOS, leading to the release of nitric oxide in endothelial cells (Tran et al., 2000). Intravenous infusion of ATP is often used to induce coronary vasodilation in patients unable to perform exercise stress tests for 201Tl scintigraphy (Faulds et al., 1991). The effect of extracellular ATP occurs via the activation of purinoceptors of the P\(_{2Y}\) subtype on endothelial cells (Yang et al., 1996). In the present study, we have shown that ATP induces an increase in nitric oxide production in BCAECs in a concentration-dependent manner. A similar finding was obtained in human coronary artery endothelial cells (Yang et al., 2000). Given that ATP may not be very stable in solution, the possibility that the effect seen may be partly due to its metabolites, e.g., ADP, cannot be excluded. The finding that ATP-stimulated rise in nitric oxide release showed the same pD\(_2\) values and the same maximal net effects in the absence or presence of cocaine suggests that cocaine has no effect on ATP-induced nitric oxide release per se but rather decreases the basal levels.

Whereas the prolonged effect of cocaine on basal nitric oxide release may be mediated in part by a decrease in eNOS expression as discussed above, the acute effect observed is likely due to a change in eNOS activity. Given that Ca\(^{2+}\) is important in the regulation of eNOS activity, we examined the effect of cocaine on intracellular Ca\(^{2+}\) concentrations. It
was not surprising that we did not see the effect of cocaine on basal intracellular Ca\(^{2+}\) levels, because of a relatively low basal Ca\(^{2+}\) level in endothelial cells and thus the difficulty to see a decrease in the basal intracellular levels due to the limitation in sensitivity of the measurement. We therefore used ATP to elevate Ca\(^{2+}\) levels and to study the effect of cocaine on intracellular Ca\(^{2+}\) mobilization. Our previous studies showed that ATP (1–300 μM) induced a concentration-dependent increase in [Ca\(^{2+}\)]\(_i\), in coronary artery endothelial cells (Yang et al., 2000). In the present study, we chose a submaximal concentration (~70% of the maximal effect) of ATP to elevate Ca\(^{2+}\). The finding that cocaine decreased intracellular Ca\(^{2+}\) concentrations elevated by ATP suggests a possible mechanism of decreased eNOS activity in the acute effect of cocaine. In addition, the cocaine-inhibited [Ca\(^{2+}\)]\(_i\) was transient and reversible by washing out the drug, indicating that the short-term effects of cocaine were not associated with permanent endothelial cell damage. Although the mechanisms for the cocaine-induced decrease in intracellular Ca\(^{2+}\) in endothelial cells are not fully understood at present, previous studies on myocytes have suggested several possible sites at which cocaine may interfere with the [Ca\(^{2+}\)]\(_i\), transient (Przywara and Dambach, 1989; Stewart et al., 1991; Huang et al., 1997): 1) inhibition of sarcolemmal Na\(^+\)/Ca\(^{2+}\) exchanger, 2) reduction of the slow inward Ca\(^{2+}\) entry via reversal of Na\(^+\)-Ca\(^{2+}\) exchange, and 3) alteration of Ca\(^{2+}\) release from the sarcoplasmic reticulum. The results of these actions of cocaine would lead to decreased Ca\(^{2+}\) entry to the sarcoplasmic reticulum and decreased Ca\(^{2+}\) load in sarcoplasmic reticulum in cardiomyocytes (Renard et al., 1994). It has been suggested that different Ca\(^{2+}\) pools may exist in a number of cells and participate in a coordinated manner in the regulation of Ca\(^{2+}\) release and subsequent Ca\(^{2+}\) wave and oscillations (Meldolesi et al., 1990; Jacob, 1991). It has been demonstrated in endothelial cells that the initial component of ATP-induced [Ca\(^{2+}\)]\(_i\), transient results from release of Ca\(^{2+}\) from endoplasmic reticulum, whereas the delayed component contributing to the plateau phase is dependent on Ca\(^{2+}\) influx from the extracellular space (Tran et al., 2000). In the present study, we have found that cocaine decreases the initial component of ATP-induced [Ca\(^{2+}\)]\(_i\), transient without affecting the plateau phase. Together, we speculate that cocaine may act through one or both of the following mechanisms: 1) inhibiting Ca\(^{2+}\) entry into the endoplasmic reticulum, resulting in a decrease in Ca\(^{2+}\) load in the endoplasmic reticulum and; 2) decreasing Ca\(^{2+}\) release from the endoplasmic reticulum.

Although the mechanisms of decreased nitric oxide production by short-term cocaine treatment are not entirely clear at present, our finding that cocaine drives eNOS redistribution and increases plasmalemmal membrane binding state of eNOS in BCAECs suggests that cocaine may decrease nitric oxide synthesis by interfering with the eNOS activation/deactivation cycle. It has been demonstrated that the dynamic equilibrium of eNOS plasmalemmal membrane targeting in cultured endothelial cells is exquisitely sensitive to changes in intracellular calcium concentrations, and translocation of eNOS from the plasmalemmal membrane to intracellular sites close to the nucleus plays a key role in the process of activation of eNOS (Michel and Feron, 1997; Prabhakar et al., 1998). The heterogeneity of cellular distribution of eNOS observed in the present study is in agreement with previous findings in bovine aortic endothelial cells (Prabhakar et al., 1998). Unlike the previous study in which ~60 to 80% untreated endothelial cells showed prominent eNOS immunostaining at the cell periphery (Prabhakar et al., 1998), the present study demonstrated most of eNOS immunostaining at perinuclear sites (~70%). This may be due in part to exposure of BCAECs to serum-free medium in the present study. Similar findings were reported in bovine aortic endothelial cells (Venema et al., 1996, 1997). Nevertheless, the present study demonstrated that short-term cocaine treatment significantly increased plasmalemmal membrane binding state of eNOS in BCAECs, compared with the untreated cells. Since the eNOS enzyme activity is suppressed by the association between eNOS and caveolin in the plasmalemmal membrane, the present finding suggests that cocaine-induced decrease in basal nitric oxide production may be mediated in part by its induction of eNOS translocation to an inactive state in BCAECs. It has been demonstrated that the equilibrium between eNOS bound to plasmalemmal caveolin and caveolin-free eNOS determines the basal component of eNOS-dependent nitric oxide release in endothelial cells (Feron et al., 1999; Feron and Kelly, 2001; Li et al., 2001). Whereas the molecular mechanism underlying the cocaine-mediated eNOS translocation remains unclear, it may be due to inhibitory effects of cocaine on Ca\(^{2+}\) mobilization as demonstrated in the present study and its effect on the dynamic heterogeneity of lipid membranes (Jorgensen et al., 1993). In addition, cocaine can increase reactive oxygen species (Moritz et al., 2003; Dietrich et al., 2005) that may contribute to uncoupling of eNOS and a decrease in bioavailable nitric oxide.

In summary, we demonstrated that both short-term and prolonged cocaine treatments decrease nitric oxide release in bovine coronary artery endothelial cells. Multiple mechanisms may be involved in the inhibitory effect of cocaine. For the short-term effect, cocaine inhibits intracellular Ca\(^{2+}\) mobilization and increases translocation of eNOS to the plasmalemmal membrane inactive state. For the long-term effect, cocaine decreases eNOS protein levels. These findings should provide a basis to understand the mechanisms underlying the recurrent of ischemia remote from acute administration and the rapid progression of coronary artery diseases in chronic cocaine abusers and may explain the acute cocaine-induced coronary vasospasm from another perspective.

References


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