Role of Mitochondrial Cytochrome c in Cocaine-Induced Apoptosis in Coronary Artery Endothelial Cells

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ABSTRACT

Cocaine induces apoptosis in coronary artery endothelial cells. Yet the cellular and molecular mechanisms are not clear. Given that cocaine has profound toxic effects on the mitochondria, the present study examined the role of mitochondrial cytochrome c in cocaine-mediated apoptosis. Using cultured bovine coronary artery endothelial cells, we found that cocaine-induced apoptosis was dose dependently inhibited by cyclosporin A with IC50 of 0.2 μM. The maximum of 65% inhibition was obtained with 3 μM cyclosporin A. Cocaine induced a translocation of cytochrome c from the mitochondria to the cytosol with a 1.8-fold increase in cytosolic cytochrome c levels, and a corresponding decrease in mitochondrial cytochrome c. In accordance with its inhibition of cocaine-induced apoptosis, cyclosporin A blocked cocaine-induced cytochrome c translocation. Correspondingly, cocaine-induced activation of caspase-9 preceded that of caspase-3. Caspase-8 was not activated. Cocaine also produced a dose-dependent decrease in Bcl-2 protein levels, but had no effect on Bax protein levels. The cocaine-induced decrease in the Bcl-2 protein was not affected by cyclosporin A but was partially blocked by caspase-3 inhibitor Ac-DEVD-CHO. Collectively, these data indicate that the release of cytochrome c from the mitochondria and the subsequent activation of caspase-9 and caspase-3 play a key role in cocaine-induced apoptosis in these cells. Furthermore, the down-regulation of the Bcl-2 protein may play an important role in cocaine-induced release of cytochrome c.

Cocaine causes coronary artery vasoconstriction and myocardial ischemia and infarction (Fraker et al., 1990; Stambler et al., 1993). Although the multifactorial effects of cocaine on the cardiovascular system often contribute to its sympathomimetic function, we have recently demonstrated that cocaine induces apoptotic cell death in human coronary artery endothelial cells (He et al., 2000). The apoptosis of endothelium has been implicated in the processes of endothelial denudation, angiogenesis, thrombosis, and atherosclerosis (MacLellan and Schneider, 1997; Haunstetter and Izumo, 1998). Cocaine-induced apoptosis of coronary artery endothelial cells was characterized by multiple morphological and biochemical features that were of typical apoptotic cell death. However, the cellular and molecular mechanisms underlying cocaine-induced apoptosis in coronary artery endothelial cells are not clear.

The signaling pathways leading to apoptosis involve the sequential activation of cysteine proteases known as caspases, resulting in protein cleavage and breakdown of DNA molecules. It has been well documented that caspase cascade involved in apoptosis includes both initiator caspases and effector caspases (Thornberry and Lazebnik, 1998). Pro-apoptotic signals activate an initiator caspase that, in turn, activates effector caspases, e.g., caspase-3, leading to apoptotic cell death. Two initiator caspases, caspase-8 and caspase-9, mediate distinct sets of death signals. Caspase-8 is activated by the death signals that bind to death receptors located on cell surfaces (Ashkenazi and Dixit, 1998). The ligands that bind to death receptors belong to the tumor necrosis factor gene superfAMILY. In contrast, caspase-9 is involved in death induced by cytotoxic agents that usually do not bind to the death receptors. Instead, they affect mitochondria and cause release of cytochrome c, which through interaction with apoptosis-activating factor-1 activates caspase-9 (Green and Reed, 1998; Zou et al., 1999). The regulatory mechanisms of cytochrome c release are not fully understood, and are likely to vary with apoptotic stimuli and cell types. It has been known that antiapoptotic members of the Bcl-2 family block cytochrome c release, whereas pro-apoptotic member Bax promotes it (Kluck et al., 1997; Yang et al., 1997; Jurgensmeier et al., 1998; Rosse et al., 1998). The expression of Bcl-2 and Bax proteins has been shown to be under physiological and pathophysiological modulation (Cook et al., 1999).

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1 This work was supported in part by National Institutes of Health Grants HL-54094 and HL-57787, a grant-in-aid from the American Heart Association (Maclellan and Schneider, 1997; Haunstetter and Izumo, 1998). The signaling pathways leading to apoptosis involve the sequential activation of cysteine proteases known as caspases, resulting in protein cleavage and breakdown of DNA molecules. It has been well documented that caspase cascade involved in apoptosis includes both initiator caspases and effector caspases (Thornberry and Lazebnik, 1998). Pro-apoptotic signals activate an initiator caspase that, in turn, activates effector caspases, e.g., caspase-3, leading to apoptotic cell death. Two initiator caspases, caspase-8 and caspase-9, mediate distinct sets of death signals. Caspase-8 is activated by the death signals that bind to death receptors located on cell surfaces (Ashkenazi and Dixit, 1998). The ligands that bind to death receptors belong to the tumor necrosis factor gene superfAMILY. In contrast, caspase-9 is involved in death induced by cytotoxic agents that usually do not bind to the death receptors. Instead, they affect mitochondria and cause release of cytochrome c, which through interaction with apoptosis-activating factor-1 activates caspase-9 (Green and Reed, 1998; Zou et al., 1999). The regulatory mechanisms of cytochrome c release are not fully understood, and are likely to vary with apoptotic stimuli and cell types. It has been known that antiapoptotic members of the Bcl-2 family block cytochrome c release, whereas pro-apoptotic member Bax promotes it (Kluck et al., 1997; Yang et al., 1997; Jurgensmeier et al., 1998; Rosse et al., 1998). The expression of Bcl-2 and Bax proteins has been shown to be under physiological and pathophysiological modulation (Cook et al., 1999).

ABBREVIATIONS: BCAEC, bovine coronary artery endothelial cell; Ac-DEVD-CHO, N-acetyl-Asp-Glu-Val-Asp-CHO; DEVD-pNA, DEVD-p-nitroanilide; IEDT-pNA, IETD-p-nitroanilide; LEHD-pNA, LEHD-p-nitroanilide; 6-CFDA, 6-carboxyfluorescein diacetate.
Although the mitochondrial/cytochrome c death pathway mediates apoptosis in response to many stimuli, its involvement in cocaine-induced apoptosis of endothelial cells has not been demonstrated. Given that cocaine has profound effects on the mitochondria and decreases mitochondrial membrane potential (Fanel et al., 1990; Yuan and Acofa, 1996), the present study was designed to test the hypothesis that cocaine activates mitochondria-mediated apoptotic pathway in bovine coronary artery endothelial cells. The specific objectives of this study were to determine 1) whether cocaine induced translocation of cytochrome c from the mitochondria to the cytosol; 2) the time courses of cocaine-mediated activation of caspase-9, caspase-8, and caspase-3; and 3) the effect of cocaine on Bcl-2 and Bax protein expression. To demonstrate whether Bcl-2 and Bax are upstream signals to cocaine-induced translocation of cytochrome c, we also determined whether cyclosporin A, which inhibits cytochrome c release, inhibited cocaine-induced changes in Bcl-2 and/or Bax proteins in the present study.

**Experimental Procedures**

**Materials.** Hoechst 33258, cocaine, cyclosporin A, PBS, annexin V-Cy3 apoptosis detection kit, and anti-actin antibody were purchased from Sigma (St. Louis, MO). Fetal bovine serum was purchased from Hyclone Laboratories (Logan, UT). Protein assay was from Bio-Rad (Hercules, CA). Purified anti-Bax and anti-cytochrome c antibodies and Ac-DEVD-CHO were from Pharmingen (San Diego, CA). Anti-Bcl-2 antibody was from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Horseradish peroxidase-conjugated anti-mouse IgG was from Amersham Life Science (Clearbrook, IL). Prestained protein molecular weight standards were from Life Technologies (Grand Island, NY). Caspase-3, 8, and 9 colorimetric assay kits were from R&D Systems Inc. (Minneapolis, MN).

**Cell Culture.** Bovine coronary artery endothelial cells (BCAECs) were obtained from Cell Applications, Inc. (San Diego, CA). Cells were grown in the complete medium of Dulbecco’s modified Eagle’s medium (Mediatech Cellgro Inc., Herndon, VA) with glucose (4.5 g/l), 15% fetal bovine serum, 100 μM penicillin, 100 μg/ml streptomycin, and were incubated at 37°C in a humidified incubator with 5% CO2, 15% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, 1.5 mM MgCl2, 1 mM ethylenediamine tetraacetic acid (EDTA), 1 mM EGTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 2 μg/ml aprotinin, 10 μg/ml leupeptin) for 30 min. To detect cytochrome c, proteins in cytosolic and mitochondrial fractions were separated as previously described (Xiao et al., 1999). Total protein was used to detect Bax and Bcl-2 expression. Protein content was determined using a standard colorimetric protein assay (Bio-Rad). The proteins were separated by 15% (Bax, Bcl-2) SDS-polyacrylamide gels, respectively. They were then transferred to nitrocellulose membranes, and incubated with primary antibodies against Bax (1:250), Bcl-2 (1:2000), and cytochrome c (1:500), respectively, in Tris-buffered saline-Tween buffer containing 4% nonfat milk. After washing, the membranes were incubated with horseradish peroxidase-conjugated anti-mouse IgG (1:2000), and visualized using an enhanced chemiluminescence detection system (Amersham Life Science). Results were quantified using a scanning densitometer (model 670; Bio-Rad). The data were normalized by actin and presented as the percentage of the control protein levels within each group.

**Caspase Activity Assay.** Activities of caspase-3, caspase-8, and caspase-9 were determined using the corresponding caspase activity detection kits (R&D Systems Inc.). Briefly, 100 μg of total cell protein was added to 50 μl of reaction buffer and 5 μl of substrates of DEVD-pNA, IETD-pNA, and LEHD-pNA, respectively. Samples were incubated at 37°C for 8 h and the enzyme-catalyzed release of pNA was quantified at 405 nm using a microtiter plate reader. At each time point of study, the values of cocaine-treated samples were normalized to corresponding untreated controls, allowing determination of the fold increase in caspase activity.

**Statistical Analysis.** Data were presented as the mean ± S.E.M. Statistical analyses were performed by one-way ANOVA followed by Newman-Keuls post tests. Differences were considered significant at *P < .05.*

**Results**

**Cocaine-Induced Apoptosis.** Figure 1 shows cocaine-induced phosphatidyserine translocation from the inner to the outer leaflet of the plasma membrane detected by the phosphatidyserine-binding protein annexin V conjugated with Cy3. Using double fluorescence staining with annexin V-Cy3 and 6-CFDA allowed us to differentiate among live, apoptotic, and necrotic cells. As shown in Fig. 1, control live cells show staining only with 6-CFDA (green, Fig. 1A). Treatment with cocaine (100 μM for 48 h) increased the number of cells double-stained with annexin V-Cy3 (cell membrane) and 6-CFDA (red and green, Fig. 1B), suggesting that these cells were undergoing apoptotic cell death. The apparent yellow fluorescence in Fig. 1B resulted from a summing of red and green fluorescence. Some cells were only stained with annexin V-Cy3 (red, Fig. 1C), suggesting necrotic cell death or postapoptotic necrosis.

Assessment of nuclear chromatin morphology by DNA-binding dye Hoechst 33258 staining using fluorescence microscopy showed condensed, coalesced, and segmented nuclei induced by cocaine (Fig. 2). As shown in Fig. 3, quantification of cocaine-induced apoptotic nuclei defined by Hoechst 33258
indicated that cocaine induced a dose-dependent increase in apoptotic cells with EC\textsubscript{50} of 50 \textmu M. The maximum of 65% was obtained at 3 \textmu M cyclosporin A.

The inhibitory effect of cyclosporin A suggested that release of cytochrome c from the mitochondria may play an important role in cocaine-induced apoptosis in BCAECs. To further test this hypothesis, we examined directly the effect of cocaine on cytochrome c translocation from the mitochondria to the cytosol in BCAECs by Western blotting. The representative Western immunoblot showed that the monoclonal antibody for cytochrome c detected a single band at expected size of 15 kDa (Fig. 5, top). After cocaine treatment, there was an increase in cytochrome c levels in the cytosolic fraction and an accordant decrease in cytochrome c levels in the mitochondrial fraction (Fig. 5, top). Quantitative densitometry for four independent experiments revealed that cocaine increased cytosolic cytochrome c levels by 1.8-fold and decreased mitochondrial cytochrome c levels by 80% (Fig. 5, bottom). As also shown in Fig. 5, cyclosporin A (1 \textmu M) inhibited the cocaine-induced translocation of cytochrome c in BCAECs.

**Cocaine-Induced Caspase Activity.** To further support the role of cytochrome c and its subsequent activation of the caspase cascade in cocaine-induced apoptosis in BCAECs, we determined the time courses of cocaine-induced activation of the protease activities of caspase-9, caspase-8, and caspase-3. As shown in Fig. 6, after cocaine treatment caspase-9 activity was increased first and reached the maximum at 6 h and continued up to 12 h. At 24 h, caspase-9 activity returned to the control levels. Caspase-3 activity gradually increased in the first 24 h and reached its peak at 48 h. In contrast, caspase-8 activity did not change significantly during the time period of study.

**Effect of Cocaine on Bax and Bcl-2 Protein Expression.** In an attempt to understand the mechanisms underlying cocaine-induced cytochrome c release, we determined the effect of cocaine on Bax and Bcl-2 protein levels by Western blot analysis. As shown in Fig. 7, the representative Western immunoblot showed that the monoclonal antibody for the Bcl-2 protein detected a single band at expected size of 29 kDa (Fig. 7, top). Cocaine (30 and 100 \textmu M, 48 h) produced a dose-dependent decrease in Bcl-2 protein levels. Quantitative densitometry for five independent experiments revealed that cocaine produced more than 50% decrease in Bcl-2 protein levels. Because Bcl-2 can undergo cleavage by activated caspases, we examined the effect of caspase-3 inhibitor Ac-DEVD-CHO on cocaine-induced reduction of Bcl-2 protein levels. As shown in Fig. 8, cocaine-induced decrease in the Bcl-2 protein was partially blocked by Ac-DEVD-CHO. The expression of the Bax protein was also detected in BCAECs and showed a significantly lower level than that of Bcl-2 (Bcl-2/Bax, 7.5 \pm 2.4). In contrast to Bcl-2, cocaine treatment did not change Bax protein levels in BCAECs (Fig. 9).
Discussion

The present study has demonstrated that cocaine causes apoptotic cell death of BCAECs through the mitochondria-mediated pathway. This conclusion is supported by the following evidence: 1) cocaine-induced apoptosis was inhibited by cyclosporin A; 2) cocaine caused translocation of cytochrome c from the mitochondria to the cytosol, which was blocked by cyclosporin A; and 3) cocaine-induced activation of caspase-9 preceded caspase-3, whereas caspase-8 was not activated. Although the precise mechanisms underlying cocaine-induced cytochrome c release in BCAECs are not entirely clear at present, the down-regulation of the Bcl-2 protein is likely to play an important role.

Cocaine-induced apoptotic cell death of BCAECs was clearly demonstrated by plasma membrane phosphatidylserine translocation and nuclear morphological changes in the present study. Similar findings were obtained in human coronary artery endothelial cells (He et al., 2000). In both human and bovine coronary artery endothelial cells, cocaine induced apoptosis starting at the concentration of 10 μM. The EC$_{50}$ of cocaine is 50 μM in these cells. Because serum levels of cocaine in active drug abusers are often >100 μM and the repeated uses of cocaine produce dose-related accumulation in serum cocaine concentration (Benowitz, 1993; Nassogne et al., 1997; Jufer et al., 1998), the pathophysiological relevance of the present finding is fully warranted. In agreement with the previous findings in the human cells (He et al., 2000), the present study demonstrated that cyclosporin A produced a dose-dependent inhibition of cocaine-induced apoptosis, suggesting that release of cytochrome c from the mitochondria plays a key role in cocaine-induced apoptosis in

Fig. 2. Cocaine-induced nuclear morphological changes in BCAECs. Cells were stained with DNA binding fluorescence dye Hoechst 33258 and nuclear morphology was examined by fluorescence microscopy in the absence (A) and presence (B) of 100 μM cocaine for 48 h. The arrows show condensed, coalesced, and segmented apoptotic nuclei. Quantitative data are shown in Fig. 3. Bar, 50 μm.
these cells. The finding of cocaine-induced cytochrome c translocation from the mitochondria to the cytosol provides a direct link between the mitochondria and cocaine-induced apoptosis in BCAECs. In accordance with the finding that it inhibited cocaine-induced apoptosis, cyclosporin A blocked cocaine-induced cytochrome c translocation in BCAECs. Similar findings were obtained in human endothelial cells in which cyclosporin A was shown to block oxidized low-density lipoprotein-induced apoptosis and cytochrome c release (Walter et al., 1998). The notion that cocaine activates the mitochondrial apoptotic pathway in BCAECs has been further supported by the time course studies of cocaine-mediated activation of caspase-9, caspase-8, and caspase-3 in the present study. The finding that cocaine-mediated activation of caspase-9 preceded that of caspase-3 clearly demonstrated...

Fig. 3. Cocaine-induced dose-dependent increase in apoptosis and decrease in cell viability in BCAECs. BCAECs were incubated with indicated concentrations of cocaine for 48 h. Cell viability was determined by trypan blue exclusion assay, and the number of apoptotic cells was determined by Hoechst 33258 fluorescence staining as described under Experimental Procedures. Data are means ± S.E.M. for three experiments.

Fig. 4. Inhibition of cocaine-induced apoptosis by cyclosporin A in BCAECs. BCAECs were incubated with cocaine (100 µM for 48 h) in the absence or presence of increasing concentrations of cyclosporin A. The number of apoptotic cells was determined by Hoechst 33258 staining as described under Experimental Procedures. Data are means ± S.E.M. for four experiments.

Fig. 5. Effect of cocaine on cytochrome c translocation in BCAECs. BCAECs were incubated with cocaine (100 µM for 48 h) in the absence and presence of cyclosporin A (CSA, 1 µM). Cytosolic and mitochondrial fractions were separated as described under Experimental Procedures. Proteins from the two fractions were separated on 15% SDS-polyacrylamide gel, and cytochrome c was detected by Western blotting using monoclonal cytochrome c antibody. Top, representative Western immunoblots. Bottom, quantitative results. Data are expressed as percentage of the control levels for four independent experiments. C, control; COC, cocaine. *P < .05 versus the control.

Fig. 6. Time courses of cocaine-mediated activation of caspase-3, caspase-8, and caspase-9 in BCAECs. BCAECs were treated with 100 µM cocaine for the time periods indicated. Caspase activities were determined as described under Experimental Procedures. At each time point, values are expressed as the percentage of caspase activities of cocaine-treated samples versus the corresponding controls. Data are means ± S.E.M. for four experiments. *P < .05 versus the control.
that caspase-9 functions as an initiator caspase in cocaine-induced caspase cascade. The activation of caspase-9 by cytochrome c and apoptosis-activating factor-1 has been well documented (Green and Reed, 1998; Zou et al., 1999). Although caspase-9 can also be activated by caspase-8 through a death receptor-mediated pathway (Ashkenazi and Dixit, 1998), the lack of effect of cocaine on caspase-8 precludes the potential activation of caspase-9 by caspase-8 and suggests that death receptor/caspase-8 pathway may not be involved in cocaine-induced apoptosis of BCAECs. In agreement with the present finding, our previous study demonstrated that cocaine-induced apoptosis of coronary artery endothelial cells was inhibited by the inhibitors of caspase-9 and caspase-3 (He et al., 2000). Taken together, these findings demonstrate that cocaine-induced apoptosis in BCAECs is mediated by the mitochondrial pathway, and the release of cytochrome c and its subsequent activation of caspase-9 and caspase-3 play a key role in cocaine-induced apoptosis.

Although the mechanisms underlying cocaine-induced cytochrome c release in BCAECs are not entirely clear at present, the finding that cyclosporin A inhibited both cocaine-induced cytochrome c release and apoptosis suggests that loss of mitochondrial membrane potential ($\Delta \psi_{\text{m}}$) may contribute to cocaine-induced release of cytochrome c. In many cells, one of the early characteristics of apoptosis is the loss of $\Delta \psi_{\text{m}}$ resulting from dissipation of the H$^+$ gradient after opening of the permeability transition pore in the inner mitochondrial membrane (Green and Reed, 1998). Cyclosporin A prevents cytochrome c release by stabilizing the mitochondrial transmembrane potential and inhibits apoptosis (Green and Reed, 1998; Jurgenenmeier et al., 1998; Marzo et al., 1998; Walter et al., 1998). It has been reported that cocaine inhibits the activity of the terminal electron transport system of the mitochondria in fetal rat heart and decreases the heart rate (Fantel et al., 1990). More recent studies showed that cocaine caused a dose- and time-dependent decrease in mitochondrial membrane potential in primary cultures of rat cardiomyocyte, and the decline of the membrane potential occurred before the manifestation of cytotoxicity shown with the exposure of cocaine (Yuan and Acosta, 1996).

Although changes intrinsic to the mitochondria are likely to ultimately mediate cytochrome c release, the present study cannot distinguish whether loss of $\Delta \psi_{\text{m}}$ causes the initial release of cytochrome c or merely amplifies the release initiated by other mechanisms. Additionally, it has been demonstrated that the opening of an inner membrane permeability transition pore is not the only mechanism mediating cytochrome c release (Green and Reed, 1998). Members of the Bcl-2 family of proteins have been demonstrated to be associated with the mitochondrial membrane and regulate its integrity (Adams and Cory, 1998). Among over 15 different proteins in the Bcl-2 family, the antiapoptotic Bcl-2 protein has been found to be associated with mitochondrial mem-
brane and to prevent both the loss of mitochondrial membrane potential and the efflux of cytochrome c. In contrast, Bax protein has a proapoptotic effect and causes release of cytochrome c. In the present study, both Bcl-2 and Bax proteins were expressed in BCAECs, with higher levels of the Bcl-2 protein. This is in contrast with the previous findings in cultured cardiac myocytes isolated from near-term fetal rats, which showed much lower content of Bcl-2 than that of Bax (Wang et al., 1998). Nevertheless, a recent study demonstrated a developmental regulation of antiapoptotic and proapoptotic proteins in rat heart such that Bcl-2 and Bcl-xL levels were sustained during development, but Bax and Bad levels were down-regulated (Cook et al., 1999). The higher ratio of Bcl-2/Bax in adult cells may be associated with the withdrawal of adult cells from the cell cycle in the perinatal period.

The present finding that Bcl-2 proteins decreased in response to cocaine suggests that the Bcl-2 protein may play a key role in cocaine-induced apoptosis of BCAECs. Although the mechanisms underlying cocaine-mediated reduction of Bcl-2 are not clear at present, we speculate that nitric oxide may be involved. We have found that cocaine inhibits nitric oxide synthesis in BCAECs (data not shown). Nitric oxide as a bifunctional regulator of apoptosis has been proposed recently (Kim et al., 1999). Depending on cell types and concentrations, nitric oxide can be a cytotoxic effector in some cells, but a protector against apoptosis in other cells, including endothelial cells (Dimmelser et al., 1997; Kim et al., 1999). It has been demonstrated that nitric oxide maintains caspase-3zymogen in an inactive form by S-nitrosylation of the catalytic-site cysteine (Mannick et al., 1999), inhibits Bcl-2 cleavage by caspase-3, and inhibits cytochrome c release (Kim et al., 1998, 1999). In the present study, we found that cocaine-induced down-regulation of Bcl-2 protein was partially blocked by caspase-3 inhibitor Ac-DEVD-CHO. However, because cyclosporin A, which blocked cocaine-induced cytochrome c release, had no effect on cocaine-induced decrease in the Bcl-2 protein, it is likely that cocaine-induced decrease in Bcl-2 is an upstream signal of cytochrome c release in BCAECs. Collectively, our data suggest that cocaine-mediated and mitochondria-independent activation of caspase-3 and cleavage of Bcl-2, probably through an inhibition of nitric oxide, may serve as a trigger that is amplified by the mitochondria/cytochrome c pathway in BCAECs. Additional experiments are needed to determine whether a reduction of nitric oxide increases caspase-3 activity and decreases Bcl-2 in BCAECs and whether a nitric oxide donor rescues cocaine-mediated apoptosis.

Unlike Bcl-2, cocaine did not change Bax protein levels in the present study. This is in contrast to our previous findings in fetal rat heart and brain in which cocaine increases Bax expression (Xiao et al., 2000a,b). Similar findings were obtained in rat heart after coronary occlusion (Liu et al., 1998). Although it is speculated that cocaine-induced decrease in Bcl-2 may cause the translocation of Bax from the cytosol to the mitochondrial membrane leading to the release of cytochrome c.

In summary, we have shown that cocaine induces a dose-dependent increase in apoptotic cell death in cultured bovine coronary artery endothelial cells. Cocaine-induced apoptosis in BCAECs is associated with the release of cytochrome c from the mitochondria into the cytosol, and the subsequent activation of caspase-9 and caspase-3. The decrease in the Bcl-2 protein in response to cocaine may play a key role in the loss of mitochondrial membrane potential and the release of cytochrome c. Although it is speculated that cocaine-induced decrease in Bcl-2 may cause the translocation of Bax from the cytosol to the mitochondria, the direct evidence remains elusive. Increased apoptosis of coronary artery endothelial cells results in endothelial dysfunction, and is likely to play a key role in cocaine-induced coronary artery vasconstriction, leading to myocardial ischemia and infarction. In addition, because phosphatidyliner is a potent surface procoagulant and it has been demonstrated that human endothelial cells with phosphatidyliner externalization during apoptosis were markedly procoagulant (Casciola-Rosen et al., 1996), cocaine-induced phosphatidyliner externalization in BCAECs may cause pathological intravascular coagulation events and impair coronary circulation, which may also contribute in part to cocaine-induced myocardial ischemia and infarction.

References


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