Role of Cardiac Nerves in the Cardiovascular Response to Cocaine in Conscious Dogs

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Background—Although the cardiovascular toxicity of cocaine is well recognized, considerable controversy remains as to the relative contribution of local norepinephrine reuptake inhibition versus central stimulatory effects of cocaine in eliciting its cardiovascular actions. The purpose of the present study was to determine the role of cardiac nerves in mediating the left ventricular (LV) and coronary hemodynamic responses to cocaine.

Methods and Results—We studied the cardiovascular response to acute cocaine administration (1 mg/kg) in 10 intact, conscious dogs and 6 dogs with ventricular denervation (VD). There were no significant differences in baseline hemodynamic parameters or plasma catecholamines between the 2 groups. In response to acute cocaine, LV and coronary hemodynamic responses were enhanced in the VD dogs. The enhanced systemic pressor and heart rate responses in VD dogs suggest that cardiac nerves mitigate the response to cocaine through ventricular mechanoreceptors rather than mediating the responses.

Conclusions—These data suggest that peripheral blockade of norepinephrine reuptake is not the principal mechanism of the acute cardiac effects of cocaine. Rather, cardiac nerves modulate the effects of cocaine through baroreflex mechanisms. Thus, individual differences in baroreflex sensitivity may explain the hemodynamic variability observed in response to cocaine. (Circulation. 2001;103:1674-1680.)

Key Words: cocaine | baroreceptors | hemodynamics

The cardiovascular toxicity associated with cocaine use remains an increasingly important public health problem.1–4 Despite the increasing recognition of cardiovascular complications referable to cocaine, the mechanisms underlying the interactions remain incompletely understood. Among the difficulties in reconciling the relationship between cocaine abuse and cardiovascular dysfunction is the relative contribution of the multiple and integrated effects of cocaine in both human and experimental animal studies. The mechanisms attributable to cocaine include stimulation of the neuronal release of catecholamines from both the central vasomotor center and the adrenal medulla.5–10 Second, cocaine is known to be a potent inhibitor of norepinephrine reuptake, which constitutes the major mechanism for modulating the activity of the agonist at the postsynaptic receptor.11–13 Third, cocaine has been shown to have direct effects to impair baroreflex functions independently of autonomic effects.14,15 Finally, cocaine has local anesthetic properties16 that constitute its most common therapeutic application. As such, the profile of the cardiovascular effects of cocaine is critically dependent on the experimental model used. In particular, anesthesia has been shown to dramatically alter the hemodynamic profile of intravenous cocaine by mitigating its central nervous system effects and highlighting its local anesthetic properties.6–17 Similarly, it has been shown that the state of resting sympathetic nervous system activation is a dominant determinant of the ultimate cardiovascular profile of intravenous cocaine administration in rats.9,18–20

Accordingly, the purpose of the present study was to determine whether or to what extent cardiac nerves are important in mediating cardiovascular consequences of cocaine. To determine this, we compared the responses to acute intravenous doses of cocaine in intact, conscious dogs and dogs that had undergone selective ventricular denervation (VD). Selective cardiac denervation eliminates the local influence of cocaine to block norepinephrine reuptake. In addition, selective VD allowed us to assess the effects on heart rate under full cardiac innervation independently from those of the ventricular responses, including contractility and coronary blood flow. Thus, if blockade of peripheral norepinephrine reuptake is the dominant mechanism responsible for the cardiovascular effects of cocaine, then the contractile and coronary vascular effects of cocaine should be attenuated in our model. Using these experimental approaches, our goal was to understand in greater detail the autonomic mechanisms responsible for the cardiovascular effects of cocaine.
Methods
Sixteen dogs of either sex weighing between 24 and 28 kg were sedated with xylazine (10 mg/kg) and anesthetized with halothane (1 to 1.5 vol%). Through an incision in the fourth intercostal space, Tygon catheters were placed in the descending thoracic aorta and left atrium, and a Silastic catheter was placed in the coronary sinus. A solid-state pressure transducer (Konigsberg Instruments) was implanted in the left ventricle (LV) through an apical approach that facilitated high-fidelity recordings of LV pressure. Transonic flow probes were placed on the proximal portion of the left circumflex coronary artery and ascending aorta for continuous measurement of coronary and aortic blood flows, respectively. All catheters were tunneled subcutaneously and externalized infracostally, after which the thoracotomy was closed in layers and the thoracic cavity was evacuated of air. All animals received analgesics as needed for the first 72 hours. Keflin (1 g IV) was administered daily for 7 days. The dogs were allowed to recover from the surgical procedure for 2 weeks, during which time they were trained to lie quietly on the experimental table in a conscious, unrestrained state. All catheters were flushed daily and filled with a 50% heparin solution to maintain patency. Animals used in this study were maintained in accordance with the guidelines of the Committee of Animals and the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (Department of Health and Human Services publication No. NIH 85-23, revised 1985). In 6 dogs, surgical VD was performed as described previously.21,22 We tested the adequacy of VD at the time of surgery by confirming the elimination of both ECG and positive inotropic responses from left ansa subclavia and thoracic vagi and positive inotropic responses from the right ansa subclavia using electrical stimulation with a 10- to 20-Hz, 5- to 10-V, 5-ms stimulus.

We determined further adequacy of the reflex stimulation of cardiac nerves in conscious dogs by documenting responses to nitroglycerin (5 μg/kg), phenylephrine (5 μg/kg), and veratrine (5 μg/kg) administered via left atrial catheter. These tests were performed 2 to 3 weeks after surgery.

Experimental Measurements
Aortic and left atrial pressures were measured with a Statham strain gauge, which was calibrated with a mercury manometer in vivo, such that a 10-mV shift was associated with a 200-mm Hg change in pressure and with aortic and left atrial pressure in vivo. The ascending aorta and left circumflex coronary artery blood flows were measured directly from the Transonic flow probes. Measurements of arterial and coronary sinus oxygen contents, hemoglobin, and oxygen saturations were measured with an IL-482 Co-Oximeter System from Instrumentation Laboratories.

Myocardial β-adrenergic antagonist binding studies were performed with 25 μL of 251I-cyanopindolol (0.10 to 1.0 nmol/mL), 25 μL of isoproterenol (100 μmol/L) or Tris buffer, and 100 μL of membrane protein (10 μg per assay) derived from sarcolemmal membrane preparations as described previously.23 The binding data were analyzed by an interactive LIGAND program,24 and a linear regression was performed on the amount bound versus bound/free ligand.

Experimental Protocol
All dogs were studied in the fully conscious state after recovering fully from surgery. Each dog received an intravenous infusion of cocaine hydrochloride (1 mg/kg over 1 minute) dissolved in saline and administered via a peripheral vein.6 Hemodynamic measurements were recorded continuously for 30 minutes in the intrinsically sinus rhythm. Plasma levels of norepinephrine and epinephrine were sampled from the arterial catheter at baseline at 5 and 30 minutes after the administration of cocaine and were measured with the use of the radioimmunoassay.24 Plasma levels of cocaine were drawn in gray-topped tubes containing sodium fluoride to inhibit plasma pseudocholinesterase activity at 2.5 and 25 minutes after cocaine was administered.26 Samples for arterial and coronary sinus oxygen content were drawn simultaneously in heparinized 3-mL syringes.27

Data Analysis
Hemodynamic data were recorded with a multichannel magnetic tape recorder and played back simultaneously on a strip-chart recorder. Continuous recordings of LV dp/dt were derived from the LV pressure signals with operational amplifiers connected as differentiators. Differentiators were calibrated directly by substituting a triangular wave signal of known slope for the pressure signal. Mean arterial pressure was derived from the use of an electronic filter applied to the phasic arterial pressure signal. Coronary vascular resistance was calculated as the quotient of mean arterial pressure and coronary blood flow and expressed in mm Hg · mL−1 · min−1. An index of myocardial oxygen consumption was calculated as the product of coronary blood flow and the arterial-coronary sinus oxygen content difference expressed as milliliters of O2 consumed per minute.

Statistical Analysis
Significant differences in the measured parameters over time, either absolute or the percent change from baseline, were assessed with a repeated-measures ANOVA. Plasma norepinephrine and epinephrine responses were compared with the use of a Student’s t test with a Bonferroni correction applied as necessary.

Results
Confirmation of VD
Figure 1 demonstrates mean arterial pressure and heart rate responses to separate intravenous infusions of phenylephrine, nitroglycerin, and veratrine. Phenylephrine increased mean arterial pressure to a comparable degree in both intact dogs and VD dogs, resulting in comparable baroreflex-mediated decreases in heart rate. Nitroglycerin caused a similar decrease in mean arterial pressure and comparable baroreflex-mediated increases in heart rate in both groups. These data demonstrate the integrity of the innervation of the sinoatrial node. However, mean arterial pressure and heart rate responses to veratrine were abolished in the VD dogs, consistent with the established site of action of veratrine on ventricular afferents. Thus, there was physiological confirmation of selective VD in dogs after surgical denervation.

Figure 2 illustrates the inotropic response to increasing intravenous doses of the endogenous neurotransmitter norepinephrine and the β-agonist isoproterenol in intact dogs and VD dogs. There was a greater (P<0.05) inotropic response to norepinephrine in VD dogs than in intact dogs, whereas the response to isoproterenol was comparable between the 2 groups. Thus, there was supersensitivity to the endogenous neurotransmitter norepinephrine but not to isoproterenol. The mechanism of the supersensitivity involved the lack of neuronal reuptake of norepinephrine in VD dogs and not a postreceptor mechanism, because the density (intact dogs, 65±6 fmol/mg protein; VD dogs, 74±8 fmol/mg protein) and affinity (intact dogs, 0.08±0.01 nmol/L; VD dogs, 0.07±0.01 nmol/L) of myocardial β-adrenergic receptors was not different between the 2 groups.

Response to Acute Intravenous Cocaine
The Table reveals the peak hemodynamic responses to acute cocaine (1 mg/kg) in intact dogs and VD dogs. There were no significant differences in baseline LV or systemic hemody-
namic parameters between intact dogs and VD dogs, with the exception that baseline LV end-diastolic pressure (LVEDP) was significantly lower and baseline heart rate was significantly higher in the VD dogs. Although baseline differences were few, there were significant differences in peak hemodynamic responses to cocaine between the 2 groups. Peak LV systolic pressure and LV dP/dt responses were significantly greater \( (P<0.01) \) in the VD dogs than in intact dogs. There were no differences in peak LVEDP or heart rate responses between the 2 groups. Peak cardiac output response was less in the VD dogs. Surprisingly, mean arterial pressure and systemic vascular resistance responses were greater in the VD dogs. The enhanced inotropic and pressor responses to cocaine in the VD dogs were evident despite the fact that there were no significant differences in either baseline or peak plasma norepinephrine or epinephrine responses to cocaine between the 2 groups. Peak plasma cocaine levels were also not different (intact dogs, 712±132 ng/mL; VD dogs, 689±176 ng/mL).

LV systolic pressure, LV dP/dt, and heart rate responses over time were both greater in magnitude and more sustained in VD dogs than in intact dogs (Figure 3). The LVEDP response was not different between the 2 groups. The time courses of mean arterial pressure and systemic vascular resistance responses were significantly greater whereas cardiac output and stroke volume response were significantly less in the VD dogs (Figure 4).

There were no significant differences in baseline coronary vascular parameters between the 2 groups, although there was greater myocardial \( O_2 \) extraction in the VD dogs (76±3%) than in intact dogs (69±2%). Peak coronary blood flow response was greater \( (P<0.05) \) in the VD dogs than in intact dogs, but there was no difference in peak coronary vascular resistance response between the 2 groups. Peak myocardial \( O_2 \) consumption response was greater \( (P<0.05) \) in the VD dogs than in intact dogs. This enhanced response in VD dogs was not accompanied by enhanced myocardial \( O_2 \) delivery, requiring greater \( O_2 \) extraction in the VD dogs (82±2%) than

**Figure 1.** Change in heart rate (left) and mean arterial pressure (right) in response to phenylephrine (5 \( \mu \)g/kg), nitroglycerin (5 \( \mu \)g/kg), and veratrine alkaloid (5 \( \mu \)g/kg) via intra-atrial injection in intact dogs and VD dogs. Responses to phenylephrine and nitroglycerin were similar, but there was neither heart rate nor mean arterial pressure response to veratrine in VD dogs, confirming physiological VD. \( ^*P<0.05 \).

**Figure 2.** Inotropic response to graded intravenous infusions of norepinephrine (left) and isoproterenol (right) in intact dogs and VD dogs. Dose response to norepinephrine was significantly greater in VD dogs \( (^*P<0.05) \) than in intact dogs. However, dose response to isoproterenol was similar, suggesting that supersensitivity was not due to enhanced myocardial \( \beta \)-adrenergic responsiveness.
in intact dogs (76±2%). Figure 5 reveals that both the magnitude and the time course of the coronary blood flow response were prolonged significantly (P<0.005) in VD dogs, in keeping with the greater myocardial oxygen requirements in the VD dogs. There was no difference in the time course of the coronary vascular resistance response.

**Discussion**

In the present study, we observed that the hemodynamic responses to cocaine (1 mg/kg) were maintained in conscious dogs with chronic ventricular denervation, suggesting that LV and coronary vascular responses to cocaine do not require intact cardiac nerves. In contrast, cardiac nerves, particularly ventricular afferents, played an important role in mitigating mean arterial pressure, systemic vascular resistance, and inotropic and chronotropic responses to cocaine, as evidenced by the enhanced and sustained responses in these parameters in the VD dogs.

Several mechanisms and sites of action have been proposed to explain the complex cardiovascular responses to cocaine, reported in both humans and experimental animals. The autonomic nervous system has been shown to

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LVP indicates LV systolic pressure; HR, heart rate; MAP, mean arterial pressure; CO, cardiac output; SVR, systemic vascular resistance; CBF, coronary blood flow; MV̇O₂, myocardial oxygen consumption; NE, norepinephrine; and EPI, epinephrine. *P<0.01 vs baseline; †P<0.05 vs intact dogs.
play a dominant role and accounts for the widely divergent cardiovascular responses to cocaine when studies are conducted under the influence of anesthesia. However, considerable controversy remains as to whether the dominant site of action of cocaine responsible for its cardiovascular effects is a result of central nervous system stimulation or a result of inhibition of peripheral norepinephrine reuptake. Gillis et al and Dickerson et al reported that cocaine methiodide recapitulates the cardiovascular responses to equimolar doses of cocaine hydrochloride in sufentanil-sedated dogs, despite the fact that the quaternary derivative does not cross the blood-brain barrier. In contrast, Schindler et al reported no cardiovascular effects of cocaine methiodide in conscious squirrel monkeys, suggesting that central nervous system stimulation was critical to the cardiovascular effects of cocaine. Similarly, Knuepfer et al demonstrated that the cardiovascular responses to cocaine in rats are mediated initially by central nervous system excitatory effects. Chiueh and Kopin demonstrated in unanesthetized rats that norepinephrine and epinephrine released in response to cocaine were the result of centrally mediated adrenal medullary discharge of catecholamines. Vongpatanasin et al and Jacobson et al demonstrated that cocaine in humans causes increases in sympathetic neural activity via central sympathetic outflow. However, this increase in sympathetic nerve activity does not exclude a role for peripheral norepinephrine reuptake inhibition in the cardiovascular effects of cocaine.

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elicited. The central stimulatory effects of cocaine were unaltered, as evidenced by the comparable increases in both circulating norepinephrine and epinephrine in both intact and VD dogs. The consistent finding that VD did not attenuate the cardiovascular responses to cocaine argues that central effects of cocaine are both necessary and sufficient to explain its cardiovascular effects. Moreover, these data suggest that the actions of cocaine to inhibit norepinephrine transport into presynaptic nerve terminals may be irrelevant to its cardiovascular effects. These findings are consistent with the observation of others 10 that alternative norepinephrine reuptake inhibitors, such as desipramine, fail to elicit the same robust hemodynamic responses observed with cocaine.

A particularly surprising finding of the present study was the observation that not only were cardiac responses preserved in the VD dogs, but these responses were enhanced. We observed for the first time that ventricular afferents play an important role in mitigating bradycardia and hypotension in response to ventricular mechanical deformation. 31–33 Their role in the cardiovascular response to cocaine is unappreciated. Prior studies have shown that cocaine blunts sinoaortic baroreflexes 14,15 but have not examined the role of ventricular cardiac afferents. These observations that relevant doses of cocaine attenuate sinoaortic baroreflexes help to explain why sinoaortic baroreflex responses were not apparent in the present study, permitting the enhanced responses observed in the VD dogs. The absence of ventricular afferents did not alter the coronary vascular response, consistent with the notion that these reflexes have minimal effects on the coronary circulation.

The absence of ventricular afferents resulted in enhanced and sustained systemic vascular resistance responses and depressed stroke volume and cardiac output. As such, impaired cardiac reflexes may constitute a risk factor associated with enhanced susceptibility to the toxicity of cocaine. Variability in cardiac baroreflex responses may also explain the differential vascular responsiveness reported by Knuepfer et al in rats. 18–20 In this case, VD dogs acted similarly to “vascular responders,” with enhanced systemic vascular resistance responses and depressed cardiac output responses, whereas intact dogs have lesser, transient increases in systemic vascular resistance and more robust increases in cardiac output. Taken together, it is conceivable that variability in the cardiovascular response to cocaine may be determined by the integrity of baroreflex buffering of the responses.

In conclusion, we report for the first time that the cardiovascular responses to cocaine in conscious dogs do not depend on the integrity of cardiac nerves. Furthermore, the cardiovascular responses to cocaine in VD dogs were enhanced in magnitude and duration, suggesting that cardiac ventricular afferents were important in buffering of the responses to cocaine. These data suggest that the cardiovascular effects of cocaine are not dependent on intact cardiac innervation. Rather, cardiac nerves, in particular ventricular afferents, appear to play an important role in mitigating rather than mediating the LV and systemic responses to cocaine.

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References


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