Role of Nitric Oxide in Cocaine-Induced Acute Hypertension
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Cocaine causes acute hypertension by blocking catecholamine reuptake. There is evidence that it also impairs the peripheral endothelial nitric oxide system, which is normally vasodilatory. We further explored the role of nitric oxide in cocaine-induced vasoconstriction in anesthetized rats, and in vitro using isolated carotid artery segments. Cocaine administered intravenously in rats increased mean arterial pressure by 30 to 40 mm Hg within 1 min. This effect was dose dependent and the maximum effect was observed at a dose of 1.25 mg/kg. The prototype catecholamine norepinephrine induced a similar increase in blood pressure. When rats were pretreated with N\textsuperscript{G} -monomethyl-L-arginine (L-NMMA, a blocker of nitric oxide) and challenged with cocaine, the increase in blood pressure was blocked by 80%, whereas pretreatment with L-NMMA did not block norepinephrine-induced vasoconstriction. Both cocaine and norepinephrine also induced an immediate vasoconstriction in isolated carotid artery preparations. The in vitro vasoconstriction induced by cocaine was blocked by pretreatment with L-NMMA, whereas L-NMMA did not block the norepinephrine-induced vasoconstriction in vitro. Furthermore, carotid artery stripped of endothelium responded to norepinephrine but failed to respond to L-NMMA or cocaine. S-nitroso-N-acetyl-D,L-penicillamine (SNAP)—a precursor of nitric oxide—stimulated nitric oxide production in control coronary artery fragments. When these fragments were incubated with cocaine there was a 20% reduction in the production of nitrite oxide. These results suggest that cocaine exerts its peripheral vasoconstriction at least in part by inhibiting local vasodilator nitric oxide. Am J Hypertens 1998;11:708–714 © 1998 American Journal of Hypertension, Ltd.

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The widespread use of cocaine as a recreational drug has stimulated renewed interest in its mechanism of action. The complications of cocaine use affect every system of the body,\textsuperscript{1–3} and its powerful vasoconstrictor action causes acute hypertension as well as perpetuating a state of chronic hypertension that in some instances could hasten the development of chronic renal failure.\textsuperscript{4} Cocaine affects the sympathetic system by blocking the reuptake of catecholamines at adrenergic nerve endings, thus potentiating the excitatory responses of sympathetically innervated structures to endogenous and exogenous catecholamines.\textsuperscript{5} In recent years local humoral factors have been found to be important in maintaining vascular tone.\textsuperscript{6} One of these factors is nitric oxide, a powerful vasodilator released by endothelial cells.\textsuperscript{7,8} Cocaine users are known to have impaired acetylcholine-induced vasoconstriction, an effect that depends on the presence of the endothelium and is mediated by nitric oxide.\textsuperscript{9} This study further

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explores the role of nitric oxide in the pathogenesis of acute hypertension induced by cocaine in rats.

MATERIALS AND METHODS

Preparation of Animals Male Sprague-Dawley rats (Harlan; 300 to 400g) were anesthetized with intraperitoneal sodium pentobarbital (70 mg/kg). The animals were placed supine on a heated plate with a soft padding. Tracheotomy was performed, and the animals were allowed to breathe spontaneously. A catheter (PE 50, 50 cm length) was placed in one of the carotid arteries and the other end was connected to a pressure transducer (Hewlett Packard, Waltham, MA) to monitor the mean arterial pressure (MAP, mm Hg). Core body temperature was maintained by a heating lamp. Another catheter was placed into one of the jugular veins for infusion of drugs. The duration between anesthesia and the start of experiment was strictly controlled in experimental and sham animals, the variation being within 5 min.

Drug Administration Rats were pretreated with a bolus of 50 mg/kg followed by a continuous infusion of 500 µg/kg/min of L-arginine (Sigma). In the sham group saline was administered according to the same protocol. After pretreatment, cocaine at 1.25 mg/mL (3.6 mmol/L) (Sigma) or NE at 10 µg/mL (0.6 mmol/L) was infused over a period of 30 sec in a volume of 0.5 mL/kg. Mean arterial pressure was recorded before and after cocaine (or NE) administration.

Measurement of Vasoconstriction in Isolated Blood Vessel A piece of carotid artery was dissected from the rat and thoroughly washed with cold Hank’s balanced salt solution (HBSS). One end of the vessel was loosely connected to the transducer to monitor intravascular pressure; the other was tied by a suture.10 After a rapid rinse in fresh HBSS the vessel was changed to a bath containing L-NMMA (2 mmol/L) and either cocaine (0.3 mmol/L) or NE (0.6 µmol/L) (in HBSS). When the treatment involved two consecutive drug treatments the vessel was shifted after the first treatment to a normal bath containing HBSS for 5 min before it was taken to the bath containing the second drug. At the end of the experiment the artery was immersed in 100 mmol/L KCl solution to ascertain its viability. The pressure transducer was adjusted to zero at the start of each measurement. The response of the vessel to each drug was studied for 5 min.

Endothelial Stripping of Isolated Carotid Artery Isolated carotid artery (~1 cm) was stripped by gently introducing a blunt steel needle presoaked in collagenease solution (10 U/mL) (Sigma) into the vessel lumen. The needle was held inside for 5 min at 37°C in HBSS bath. After this procedure the needle was withdrawn and the vessel flushed with fresh HBSS and held in HBSS for 10 min before experimentation.

Assay for Nitric Oxide Production in Arterial Vessel Fragments Isolated vessels from the carotid and thoracic aorta were cut into small pieces and thoroughly washed in HBSS. The chopped tissue was divided equally into three parts by wet weight and placed in two vials each containing serum-free HBSS and either SNAP (S-nitroso-N-acetyl-D,L-penicillamine, 10 µmol/L), or SNAP plus cocaine (0.3 mmol/L), respectively, and incubated at 37°C for 1 h. The vials were centrifuged for 10 min at 1000 g, and supernatants were collected for nitrite assay by the Griess method.13 Blanks consisted of SNAP in HBSS and SNAP plus cocaine in HBSS. Both blanks were similar, and contained measurable amounts of nitrite but less than 10% of nitrite obtained from tissue + SNAP. The blank value was subtracted from the experimental results for nitrite determination.

Data Collection The maximum change of the absolute MAP within 1 to 5 min after administration of drugs in experimental and sham groups was compared and analyzed statistically. The data were expressed as mean ± SE. Comparisons were made between experimental treatment and appropriate controls by one-tailed Student’s t test. A P < .05 was considered significant in all statistical tests and indicated by an asterisk in the figures.

RESULTS

Acute Hypertension Induced in Rats by Cocaine Cocaine administered intravenously in anesthetized rats increased MAP by 30 to 40 mm Hg within 1 min. By 5 min the MAP returned to normal. In the next 30 min the pressure dropped to 10 to 15 mm Hg below normal before returning again to baseline. Figure 1A shows the typical MAP changes after injection of cocaine. There was a considerable variation in the basal blood pressure of rats, but the immediate hypertensive response to cocaine was always elicitable. The mechanism of the prolonged depressor response after the pressor effect is not understood but it has been noted in previous studies.14 The acute hypertensive effect of cocaine was dose dependent and the maximum effect was observed at the dose of 1.25 mg/kg (data not shown). Based on these data, the dose of 1.25 mg/kg was chosen for all subsequent experiments. The chosen dose is in the mid-range of the human dose15 and very comparable to doses used in experimental studies.14,16–19

Acute Effects of NE When a standard dose of NE was administered intravenously in anesthetized rats,
the blood pressure changes were similar to those induced by cocaine (Figure 1B). After the peak hypotension there was a prolonged period of hypotension (>25 min) before the pressure returned to normal.

**VASOCONSTRICTIVE EFFECTS OF COCAINE AND NE IN THE ISOLATED CAROTID ARTERY PREPARATION**

Both cocaine and NE induced an immediate vasoconstriction in the isolated carotid artery after the vessels were moved from the normal bath to the one containing the drugs (Figure 1C, D). As in the in vivo study, the response lasted for 1 min, after which the vessel returned to its normal state. The preparations were tested for their viability after the experiment by immersing them in a depolarizing solution of KCl. As shown in the figures the preparations were viable by appropriately responding to the KCl with vasoconstriction.

**Effect of L-NMMA on Cocaine-Induced Acute Hypertension**

In rats given a bolus and then continuously infused with L-NMMA, there was an immediate increase in MAP (20 to 30 mm Hg), which returned to baseline in 20 to 40 min. When rats were injected with the test dose of cocaine at this time, the blood pressure increased only slightly, amounting to an 80% decrease in the normal response of cocaine (Figure 2, top). Similar experiments were performed in another group of rats to test for the response of NE in L-NMMA-pretreated animals. As also shown in the same figure
the response to NE was comparable to that of controls, despite the presence of L-NMMA, indicating that the inhibition of the normal cocaine response by L-NMMA was a specific effect.

Effect of Pretreatment With L-NMMA on Cocaine-Induced Vasoconstriction in Isolated Carotid Artery Preparation The blocking effect of L-NMMA observed in the intact rat was also tested in vitro. As
shown in Figure 2 (bottom) L-NMMA completely inhibited the cocaine-induced vasoconstriction, but it did not affect the NE-induced vascular contraction. These data support the in vivo finding that the nitric oxide system has a role in the cocaine-induced hypertensive response.

Cocaine-Induced Vasoconstriction in Endothelial-Stripped Carotid Artery Preparation The stripping technique was standardized previously on several vessels by sequentially testing them for a negative vasoconstrictive response to L-NMMA and a positive response to NE (data not shown). Once the technique was reproducible in our hands, the effect of L-NMMA, cocaine, and NE were tested in separate sets of vessel preparation. The data in Figure 3 show that the L-NMMA and cocaine effect were unelicitable in stripped vessels, which retained the normal vasoconstrictive response to NE, further supporting the important role of the endothelial nitric oxide system in the cocaine effect.

The Effect of Cocaine in the Generation of Nitric Oxide in Carotid Artery Fragments On the basis of the results presented thus far we hypothesized that cocaine could cause vasoconstriction at least in part by inhibiting the production of nitric oxide in the endothelium. This was tested in the next group of experiments by directly measuring nitrite (a catabolite of NO) in arterial fragments stimulated by SNAP in the presence of cocaine. SNAP is a donor of NO but requires enzymatic biotransformation to generate NO20, therefore, it increases the basal level of NO availability. The data shown in Figure 4 showed that cocaine was effective in inhibiting the induction of nitric oxide by 20%.

DISCUSSION

In this article we investigated the role of the endothelium-derived vasodilator nitric oxide in cocaine-induced acute hypertension. Our results showed that nitric oxide synthase (NOS) inhibitors blocked the acute hypertensive effect of cocaine in intact animals and also in isolated carotid artery segments. Stripping the endothelial layer from isolated arteries abolished the vasoconstrictive effect of cocaine but maintained that of NE. This would suggest that the action of cocaine is mediated at least in part by inhibiting nitric oxide. To confirm this we incubated isolated artery fragments with cocaine in the presence of SNAP, a stimulator of nitric oxide.20 In this condition the release of nitric oxide was reduced, showing that somehow cocaine interfered with the synthesis or release of nitric oxide. In trying to explain these observations we found that previous studies had focused on the sympathomimetic,14,16 calcium channel-related,17,18 and local anesthetic properties of cocaine19 to explain the vasoconstrictive effects. Our results suggest an additional pathway of cocaine action through the modulation of the intrinsic endothelial nitric oxide system.

FIGURE 4. Effect of cocaine on the production of nitrite (a catabolic product of nitric oxide) in carotid arterial fragments. Limit bars show standard errors (n = 6 for each treatment). * indicates significant statistical difference (P < .01) compared to control group. The fragments were incubated in SNAP, an inducer of nitric oxide. In presence of cocaine there was a 20% inhibition in the production of nitric oxide.
Because cocaine acts by preventing catecholamine reuptake, it was unclear why the effect of cocaine was totally prevented by L-NMMA. Our results would suggest that nitric oxide is also involved in the release or maintenance of catecholamine levels at the peripheral nerve terminals. In the central nervous system, nitric oxide has been shown to augment the release of epinephrine at the presynaptic terminal by a retrograde pathway. It could be speculated that pretreatment with L-NMMA blocks nitric oxide production leading to depletion of catecholamine at the presynaptic junction. This could explain the lack of sympathomimetic effect of cocaine in the presence of L-NMMA. As expected the reactivity toward exogenous NE was not altered by L-NMMA.

Although it is accepted that cocaine acts mainly through the sympathetic system, there are other effects that may be mediated through different pathways. Some of these pathways may be exerted through the endothelium. In one study in pigs when the left descending coronary artery was denuded of the endothelium, it failed to vasoconstrict upon injection of cocaine. In another study in humans, it was found that acetylcholine-induced vasorelaxation in the forearm was significantly impaired in cocaine abusers compared to control subjects, suggesting that cocaine interfered with endothelium-dependent vasorelaxation. It was not clear from these studies whether these cocaine effects were due to the specific inhibition of nitric oxide release caused by cocaine. Our experimental results, showing a partial inhibition of endothelial nitric oxide release by cocaine, provides a mechanistic basis for these previous observations.

There is a growing body of literature on the newly discovered nitrergic neuronal pathways, which involve nitric oxide as the neurotransmitter. Both inhibitory and excitatory nitrergic pathways have been described. In several studies pretreatment of animals with L-NAME blocked the convulsive and locomotor actions of cocaine, suggesting the use of these pathways by cocaine. However, these studies fell short of showing a direct effect of cocaine on nitric oxide generation. Our study is the first report of the effect of cocaine on endothelial nitric oxide, which has an important role in the normal maintenance of blood pressure.

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