Catecholamines, Cocaine Toxicity, and Their Antidotes in the Rat[1] (43177)

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Abstract. **Acute lethal cocaine intoxication in the rat induces significant increases of plasma dopamine, norepinephrine, and epinephrine concentrations associated with cardiac functional and morphologic changes. Nitrendipine (a calcium channel antagonist) administered 5 min following cocaine administration lowers catecholamine concentration and restores cardiovascular function to normal, while preventing lethality, and so does enalaprilat (an enzyme-converting inhibitor) administration with diazepam. Cocaine cardiac toxicity in the rat appears to be associated with a significant stimulation of the sympathoadrenal and a sustained elevated plasma concentration of epinephrine. The renin angiotensin system also appears to be activated.** [P.S.E.B.M. 1991, Vol 196]

Cocaine inhibits norepinephrine reuptake in tissues (1, 2) and releases norepinephrine and epinephrine from the adrenal medulla (3, 4). Cocaine administration is also associated with elevated dopamine, norepinephrine, and epinephrine concentrations in the squirrel monkey (5) and in the dog (6). In animal preparations, functional and morphologic cardiovascular changes are also observed following cocaine administration (7), and these mimic those induced by norepinephrine or by angiotensin II (8, 9). In man, cocaine self-administration produces elevated blood pressure and tachycardia (10) and has been related to myocardial infarction (11), and subarachnoidal hemorrhage (12) as well as myocardial band necrosis (13). Experimental studies demonstrate that a dihydropyridine, nitrendipine, will prevent the cardiovascular changes and cardiac lesions induced by lethal doses of cocaine administration to the restrained instrumented rat (14, 15). Other studies show that nitrendipine is an effective antidote against lethal cocaine intoxication (16), a property shared by enalaprilat (the angiotensin-converting enzyme inhibitor) when administered with diazepam (17). These compounds, administered to the rat 5 min after a lethal dose of cocaine, will correct the central nervous system and cardiovascular anomalies induced by this drug. It was also reported that nimodipine, another calcium channel antagonist, administered to the squirrel monkey, will prevent or mitigate the changes in heart rate and blood pressure, as well as the increments in plasma catecholamines produced by the intravenous administration of small doses (0.5–2 mg/kg) of cocaine (5). The purpose of the present studies was 2-fold: (i) to investigate the effects of cocaine on plasma catecholamine concentrations in rats administered intraperitoneally 60 mg/kg of the alkaloid, a lethal dose which will produce marked cardiovascular and central nervous system disturbances; (ii) to test the effects of antidotes on these cocaine-induced hormonal changes. The antidotes selected for this purpose, nitrendipine and enalaprilat (administered with diazepam) had been tested previously under such conditions, and had prevented the lethal outcome of the treated animals (14–17).

Materials and Methods

Sprague-Dawley rats with an average weight of 380 ± 19 g were fitted (under ether and local anesthesia) with catheters in the caudal and right carotid arteries. The carotid catheter was tunneled under the skin to the posterior part of the head and filled with heparinized solution. The caudal artery catheter was connected to a microinfusion pump and to a recorder for on-line recording of arterial pulse pressure which was analyzed and processed by a microcomputer; measurements of heart rate and pulse pressure were continuously dis-

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played and printed out every 30 sec. This model was described previously (15). After 4 hr, the animal was placed in a restraining grid. Four groups, with six rats in each group, were studied according to the protocol described in Figure 1. Animals in Group 1 were administered 0.6 ml of saline intraperitoneally, while those in Groups 2, 3, and 4 were given 60 mg of cocaine intraperitoneally in 0.6 ml of saline solution. Five minutes after the intraperitoneal administration of saline or cocaine, the rats were treated as follows: those in Groups 1 and 2 were given an infusion of saline at the rate of 15 μl/min. Animals in Group 3 were administered a loading dose of 7.2 μg of nitrendipine followed by a slow infusion of 1.2 μg/kg/min. Rats in Group 4 were given a 50-μl bolus of 0.3 mg of enalaprilat/kg followed by a 50-μl bolus of 0.7 mg of diazepam/kg. The dose of antidotes administered corresponds to those used in other experiments and which prevented lethality in rats given the same lethal dose of cocaine (60 mg/kg) (16, 17).

Two successive 0.8-ml samples of blood were taken from the carotid catheter: Sample A, 4 min after intraperitoneal administration of saline or cocaine, and Sample B, 5 min after treatment of the animal with saline or the selected antidotes. The blood withdrawn caused a fall in blood pressure, which was corrected by the rapid replacement of blood loss with the same volume of dextran. Blood samples were immediately refrigerated and centrifuged, and the plasma was separated and frozen. The radioenzymatic method of Peuler and Johnson (18), as modified by the Cat A kit instructions supplied by Amersham-International 1986, was used for catechol measurements.

Animals administered cocaine without treatment with nitrendipine or enalaprilat plus diazepam succumbed within minutes after the last sample was withdrawn. Treated animals were sacrificed 40 min after the last sample. Macroscopic examination of the hearts of all animals was performed.

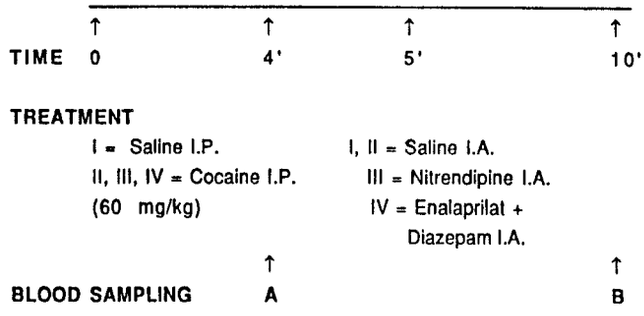

Figure 1. Schedule of treatment and blood sampling in four groups (Groups 1–4) of rats (six animals in each group). Animals were first administered intraperitoneally: in group 1, 0.8 ml of saline; in Groups 2–4, 60 mg/kg cocaine in 0.8 ml of saline. Five minutes after, animals in groups 1 and 2 were administered saline intra-arterially. Those in Groups 3 and 4 were given either nitrendipine or enalaprilat intra-arterially followed by diazepam. Blood sample A was taken 4 min after the start of the experiments; Sample B, 5 min after administration of saline (Groups 1 and 2) or antidote (Groups 3 and 4).

Results

Sequential measurements of catecholamines in plasma samples (A and B) were analyzed according to the paired *t* test (Table I). Plasma epinephrine and norepinephrine concentrations from animals administered saline or cocaine were significantly higher in Sample B than in Sample A. This difference was not present in the plasma of animals first administered cocaine and subsequently treated with antidotes. Analysis of variance of catecholamine concentration of Sample A was performed in all groups treated with cocaine (Groups 2–4). No significant difference was recorded (Table II). Catecholamine concentrations of samples from saline control (Group 1), and pooled cocaine administered (Group 2), were compared using the protected least significant difference (PLSD) test; except for dopamine, they were significantly higher in all samples of animals administered cocaine. A one-way analysis of variance was performed on Sample B in each group (Table III). The dopamine concentration was significantly different between saline control (Group 1) and cocaine administered (Group 2). The epinephrine concentration was significantly greater in Group 2 than 1 and significantly lower in Groups 3 and 4 compared with Group 2.

As previously reported (1, 3), rats administered cocaine had increases in blood pressure, arrythmias, and, at autopsy, myocardial lesions. Administration of the previously defined antidotes to cocaine restored blood pressure and cardiac rhythm to normal, and prevented the occurrence of morphologic changes in the heart (15, 16).

Discussion

Elevated plasma catecholamines in the restrained rat have been reported by other investigators (19). In the present studies, the sampling of blood and resulting fall in blood pressure should have also contributed to the elevation in plasma catecholamine observed in Sample B of the control rat administered saline. In animals administered cocaine (Groups 2–4), the concentration of epinephrine in Sample A was higher than in control group (Group 1). After administration of antidotes (Sample B), there were no significant differences between the saline control and antidote-treated animals (Table II). As previously reported, cocaine administration to the squirrel monkey (5) and the dog (6) results in higher plasma concentrations of epinephrine than of norepinephrine. In the latter study, epinephrine levels were strongly correlated with the hemodynamic response, whereas plasma cocaine levels were not. However, in cocaine-intoxicated human subjects, the concentration of plasma norepinephrine higher

Table I. Plasma Catecholamine Concentration in Four Groups of Animals Treated as Indicated in Figure 1[a]

Catecholamine plasma concentration (pmol/ml)	Group 1: saline (A) + saline (B)	Group 2: cocaine (A) + saline (B)	Group 3: cocaine (A) + nitrendipine (B)	Group 4: cocaine (A) + enalaprilat + diazepam (B)
A:Dopamine	91 ± 30	155 ± 22	185 ± 129	226 ± 222
B:Dopamine	100 ± 42[b]	223 ± 82[c]	188 ± 58[b]	239 ± 114[b]
A:Epinephrine	364 ± 83	579 ± 255	775 ± 778	572 ± 419
B:Epinephrine	1368 ± 753[d]	2430 ± 649[e]	1318 ± 636[b]	1264 ± 883[b]
A:Norepinephrine	323 ± 195	603 ± 121	673 ± 380	776 ± 508
B:Norepinephrine	704 ± 201[f]	1538 ± 702[g]	1223 ± 551[b]	1475 ± 700[b]

[a] Sample A was taken 4 min after intraperitoneal administration of saline (Group 1) or of cocaine (Groups 2–4). Sample B was taken 5 min after intravenous administration of saline (Groups 1 and 2) or of nitrendipine (Group 3) or of enalaprilat + diazepam (Group 4). Statistical analysis, paired *t* test.
[b] NS.
[c] $P < 0.06$.
[d] $P < 0.015$.
[e] $P < 0.002$.
[f] $P < 0.023$.
[g] $P < 0.04$.

Table II. Analysis of Variance of Sample A in Groups (Groups 2–4) Treated with Cocaine ($F = 1.85$; $P = 0.33$, NS) and Analysis of Variance of Sample A between Saline Control (Group 1) and Pooled Cocaine-Administered Rats (Groups 2–4, Fisher Test PLSD)

Dopamine	$F = 1.47$, NS
Epinephrine	$F = 5.60$, $P < 0.027$
Norepinephrine	$F = 5.50$, $P < 0.029$

Table III. One-Way Analysis of Variance of Sample B

Dopamine ($F = 3.66$; $P < 0.031$, S)
- Groups 1 and 2 (Fisher test PLSD), $P \leq 0.0151$, S
- Groups 1 and 4 (Fisher test PLSD), $P \leq 0.0068$, S
- Groups 1 and 3, 2, and 4, 4 and 3, 3 and 2, NS

Epinephrine ($F = 3.36$; $P < 0.0406$, S)
- Group 1 and 2 (Fisher test PLSD), $P < 0.025$, S
- Groups 2 and 3 (Fisher test PLSD), $P < 0.0228$, S
- Groups 2 and 4 (Fisher test PLSD), $P < 0.0135$, S
- Groups 3 and 4, 1 and 3, 1 and 4, NS

Norepinephrine ($F = 2.59$; $P \leq 0.08$, NS)
- Group 1 (control)—1 intraperitoneal injection of saline + 1 intravenous injection of saline
- Group 2—1 intraperitoneal injection of cocaine + 1 intravenous injection of saline
- Group 3—1 intraperitoneal injection of cocaine + 1 intravenous injection of nitrendipine
- Group 4—1 intraperitoneal injection of cocaine + 1 intravenous injection of enalaprilat and diazepam

than that of epinephrine has been observed (20). Drug treatment of the intact rat with doses of nitrendipine large enough to significantly decrease blood pressure will tend to increase catecholamines by a central baroreflex-mediated effect. If cocaine releases catecholamines as suggested in this and other studies, a stimulation of the renin-angiotensin system may ensue. Conversion enzyme inhibitors may directly (by prejunctional effect) and indirectly, by curtailing production of angiotensin II, reduce the release of noradrenaline in the vessel wall. Converting enzyme inhibitors may also directly reduce the responsiveness of vascular smooth muscle to vasoconstrictor stimuli (such as adrenoceptor activation) (21). The effect of drugs like enalaprilat is to decrease both sympathoadrenal outflow and the stimulation of the renin-angiotensin system. Enalaprilat has primarily a peripheral effect on the renin-angiotensin system, since it does not cross rapidly the blood-brain barrier. Diazepam, which controls the lethal convulsions induced by cocaine (22), when administered in association with enalaprilat, appears to be an effective antidote to cocaine in preventing its central and cardiac peripheral ontoward effects. Untreated animals with the highest catecholamine levels presented gross acute myocardial damage. These lesions were not present in the animals treated with antidotes which restored catecholamine concentrations to the same level as those of restrained animals given only saline. Others have reported experimental production of myocardial lesions following norepinephrine, epinephrine, or angiotensin administration (8, 9, 23), which were similar to those described after cocaine intoxication (14, 15). Arrhythmias, myocardial infarction, and a significant incidence of myocarditis and carciomyopathy have also been reported in patients with pheocromocytoma (24). It is noteworthy that cocaine-intoxicated animals treated with nitrendipine or enalaprilat-diazepam did not present the agitation, tremors, and cocaine-induced convulsions which might be related to an increased release of dopamine, norepinephrine, and angiotensin in the brain.

Central and peripheral stimulation by cocaine of

the sympathetic and renin-angiotensin systems appears to be a major component of the acute toxicity of this alkaloid, which products the uncontrolled escape of physiologic mechanisms useful in normal regulation (25). Antidotes to cocaine will correct this drug-induced deregulation. The possible contribution of the local anesthetic property of cocaine in the genesis of cardiac cocaine toxicity remains to be established.

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