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Molecular characteristics of cocaine-induced cardiomyopathy in rats

Sophie Besse [a], Patrick Assayag [b], Colette Latour [c], Chantal Janmot [d], Valérie Robert [a], Claude Delcayre [a], Gabriel Nahas [c,e], Bernard Swynghedauw [a,*]

[a] *Institut National de la Santé et de la Recherche Médicale-Unité 127 and Institut Fédératif de Recherche Circulation, Hôpital Lariboisière, 41 Boulevard de la Chapelle, 75010 Paris, France*
[b] *Service de Cardiologie B, Hôpital Bichat, 75018 Paris, France*
[c] *Laboratoire de Toxicologie Cellulaire, Hôpital Fernand Widal, 75010 Paris, France*
[d] *URA-CNRS 1131, Université Paris-Sud, 91405 Orsay, France*
[e] *Department of Anesthesiology, New York University Medical Center, New York, NY 10016, USA*

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Abstract

Cocaine abuse induces severe cardiomyopathy. To investigate the molecular effects of acute and prolonged administration of cocaine, mRNAs encoding markers of either mechanical overload, as atrial natriuretic factor (ANF) and α- and β-myosin heavy chains, or fibrosis as type I and III procollagens, were quantitated in the left ventricle of rats 4 h after one injection of cocaine (40 mg/kg, $n = 7$), or 14 ($n = 15$) and 28 days ($n = 10$) after chronic infusion of cocaine (40 mg/kg per day). Plasma cocaine and benzylecgonine concentrations were both significantly augmented during the infusion while plasma levels of triiodothyronine and thyroxine were lowered. Acute injection of cocaine induced ANF gene expression. Cocaine treatment during 28 days resulted in left ventricular hypertrophy (+20% after 24 days, $P < 0.05$) with normal blood pressure, associated with an accumulation of mRNAs encoding ANF and type I and III collagens (+66% and +55%, $P < 0.05$). Such a chronic treatment also induced a shift from the α- to the β-myosin heavy chain gene expression (−40% and +50%, $P < 0.05$). In conclusion, cocaine activates markers of both hemodynamic overload and fibrosis. Such an activation may result from direct and/or indirect effects of the drug such as myocardial ischemia, mechanical overload and/or hypothyroidism.

Keywords: Cocaine; Molecular biology; Collagen; Myosin; Atrial natriuretic factor; Left ventricular hypertrophy; (Rat)

1. Introduction

The epidemic increase in cocaine abuse during the last decade has focused public and scientific attention on the cardiovascular consequences of unrestricted use of this drug. Experimental acute cocaine administration depresses myocardial contractility, both in vivo and in vitro, increases blood pressure and induces coronary artery vasoconstriction and myocardial ischemia (Fraker et al., 1990; Pagel et al., 1992; Stambler et al., 1993). In addition, chronic cocaine abuse has been reported to be associated with sudden death, arrhythmias, myocardial infarction (Hollander, 1995) and cardiomyopathies including left ventricular hypertrophy (Virmani et al., 1988; Brickner et al., 1991).

Left ventricular hypertrophy resulting from mechanical overload in both humans and animals models is currently associated with myocardial changes in expression of genes encoding cardiac hormones such as atrial natriuretic factor (ANF) (Mercadier et al., 1989; Feldman et al., 1993; Robert et al., 1994), contractile proteins of the myocyte such as myosin heavy chain (Lompré et al., 1979; Feldman et al., 1993) or extracellular matrix components such as collagens (Bhambi and Eghbali, 1991; Robert et al., 1995).

While acute cocaine administration is associated with activation of several immediate early genes in brain (Hope et al., 1992), cardiac alteration of gene expression remains to be investigated in cocaine-induced cardiomyopathy. Indeed, there are few experimental data relating prolonged cocaine administration to chronic cardiovascular disease and the physiopathology of cocaine-induced cardiomyopathy remains poorly understood.

The goal of this work is to investigate the effects of

* Corresponding author. Tel.: (33-1) 4285-8065; Fax: (33-1) 4874-2315.

acute or prolonged administration of cocaine on (1) ANF mRNAs as a marker of acute mechanical overload (Mercadier et al., 1989; Feldman et al., 1993; Robert et al., 1994), (2) α- and β-myosin heavy chain mRNAs as markers of prolonged mechanical overload (Lompré et al., 1979; Izumo et al., 1987; Feldman et al., 1993; Besse et al., 1993), and (3) type I and III procollagens as markers of active fibrosis (Bhambi and Eghbali, 1991; Robert et al., 1994) in order to precise the genesis of the cocaine-induced cardiomyopathy.

The main finding was that cocaine administration in rats activates molecular markers of both chronic mechanical overload and fibrosis, suggesting that these two mechanisms play a role in cocaine-induced cardiomyopathy.

2. Material and methods

2.1. Experimental protocol

Male Sprague Dawley rats from IFFA Credo (Lyon), were submitted to acute or prolonged cocaine treatment. The dose of 40 mg/kg per day is well tolerated and, when related to body surface, corresponds to a dose of 10 mg/kg per day in man, a dose currently used by cocaine abusers. Cocaine abusers are used to consume 1 to 2 g daily, either sniffed, injected or smoked. Animals were fed ad libitum with M20 pellets (Extralabo). All experiments on living animals (executive order of French Ministry of Agriculture No 87-848, authorization 006083) were in accordance with the Declaration of Helsinki and the internationally accepted principles in the care and use of experimental animals.

For acute administration, male rats weighing 320–340 g were injected intraperitoneally with a single dose of cocaine hydrochloride (40 mg/kg) or isotonic saline solution (NaCl) and sacrificed 30 min, 1, 2 or 4 h later. For prolonged administration, male rats weighing 220–240 g were randomly divided into 4 groups. After anesthesia with pentobarbital (60 mg/kg i.p.), an osmotic minipump (Alzet, Charles River) containing cocaine hydrochloride (200 mg/ml at pH 4.5) or isotonic saline solution (0.9% sodium chloride at pH 7.4) was intraperitoneally implanted to continuously deliver cocaine (40 mg/kg per day i.p.) or saline solution for 14 or 28 days. Animals were fed ad libitum with M20 pellets (Extralabo).

After 14 or 28 days of treatment, animals were anesthetized (pentobarbital: 60 mg/kg i.p.) and the heart was removed, rinsed in icecold saline solution, and blotted dry. The left ventricle including the septum and right ventricle were weighted, frozen in liquid nitrogen and stored at -70°C until used. Osmotic minipumps, removed after treatment, still contained 200 mg/ml cocaine at pH 4.5 and routine examination showed no necrotic areas around the pump.

Using an identical protocol, the hemodynamics were investigated under anaesthesia in four groups of rats administered with cocaine or saline for 14 or 28 days. Blood pressure and heart rate were measured by a catheter, inserted in the caudal artery and connected to a pressure transducer (P23dB, Statham) (Trouvé et al., 1987, 1996) and analyzed by a microcomputer.

2.2. Plasma determinations of cocaine and hormone concentrations

In a separate previously published (Arnaoudov et al., 1995) series of experiments, 15 animals were fitted with i.p. osmotic pumps delivering 40 mg cocaine/kg per day. Three animals were killed on days 2, 6, 13, 19 and 28. Their chests were opened and hearts punctured for the sampling of blood which was processed and analyzed by high performance liquid chromatography for plasma cocaine and benzylecgonine concentrations.

Plasma free triiodothyronine (T3) and thyroxine (T4) (T3 and T4 INCSTAR kits, Stillwater, MN, USA), cortisol (INCSTAR kit), aldosterone (Abbott Laboratories kit, Abbot Park, IL, USA) and active renin (renin active Pasteur CT kit, ERIA Diagnostics Pasteur, France) concentrations were performed by radioimmunoassay.

2.3. RNA extraction. Northern and slot blots analysis

Total RNA from individual left ventricles was isolated according to Chomczynski and Sacchi (1987) and resuspended in 0.1% sodium dodecyl sulfate (SDS). For Northern blot analysis, 20 μg of total RNA were denatured in 50% formamide, 2.2 M formaldehyde and $1\times$3-[*N*-morpholino] propane sulfonic acid (MOPS) buffer (pH 8.0), size-fractionated on 1% agarose gels and then transferred to nylon Hybond N membrane (Amersham). For slot blot analysis, denatured total RNAs (1, 2, 4 and 10 μg) were applied directly to nylon Hybond N membranes. All blots underwent ultraviolet irradiation to covalently link the RNA samples.

Northern blots containing RNA samples from the left ventricle of acutely treated rats were sequentially hybridized with a 40-mer oligonucleotide complementary to nucleotides 393–432 in the 3′ translated region of the rat ANF mRNA (Mercadier et al., 1989) and a 24-mer oligonucleotide complementary to nucleotides 1046–1070 of the rat ribosomal 18S RNA (Pasteur Institute, Paris) (Besse et al., 1993). Northern and slot blots were sequentially hybridized with a synthetic 42-mer oligonucleotide probe complementary to nucleotides 1286–1326 from the 3′ end of the α-myosin heavy chain DNA (Delcayre et al., 1992), a synthetic 42-mer oligonucleotide probe complementary to nucleotides 1286–1326 from the 3′ end of the β-myosin heavy chain DNA (Delcayre et al., 1992), an α1(I) procollagen cDNA encoding for type I collagen (Besse et al.,

Table 1
Plasma levels of cocaine and benzoylecgonine during chronic administration of cocaine

Day	Plasma cocaine (ng/ml)	Plasma benzoylecgonine (ng/ml)
2	755 ± 167	3366 ± 486
6	784 ± 95	3320 ± 1029
13	1605 ± 74	6257 ± 624
19	3311 ± 380	6095 ± 646
28	3699 ± 494	6083 ± 1053

Cocaine was administered continuously using an osmotic minipump (40 mg/kg per day, i.p.). Mean ± S.D., $n = 3$ for each determination.

1994; Robert et al., 1994), an α1(III) procollagen cDNA encoding for type III collagen (Besse et al., 1994; Robert et al., 1994), the 18S rRNA oligonucleotide probe described above and finally a 25–30 mer oligo d(T) (Pharmacia, Les Ulis) which hybridizes to the poly(A^+) tails of the total mRNA population. The last two probes were used to normalize the measurements on slot blots (Besse et al., 1993, 1994; Assayag et al., 1997). The cDNA probes were labeled by random priming with [α-^{32}P]dCTP with a Rediprime Kit (Amersham) and the synthetic oligonucleotides with T4 polynucleotide kinase and [γ-^{32}P]ATP. Dehybridizations were performed in boiling 1× standard saline citrate, 0.1% SDS for 5 min and blots were exposed to verify the dehybridization (Besse et al., 1994). The washed blots were exposed to X-ray films (Hyperfilm Amersham) with Quanta III intensifying screens for 16 to 96 h at −70°C, and the relative level of each mRNA species was determined by scanning densitometry within the linear response range of the X-ray films. The densitometric scores of α- and β-myosin heavy chain and type I and III collagen mRNAs were normalized for both 18S rRNA and poly(A^+) mRNA. The densitometric scores of ANF mRNAs were normalized for 18S rRNA.

2.4. Statistical analysis

Values were expressed as mean ± S.E.M. The statistical significance of differences between the various groups was determined by one-way analysis of variance and group-to-group comparisons were made by the Scheffé F test when the number of rats (n) was < 7, or by two-tailed unpaired Student's t-test when $n \geq 7$. A P value < 0.05 was considered to be statistically significant.

3. Results

3.1. Plasma levels of cocaine

The plasma concentration of cocaine has previously been determined in the same experimental conditions (Arnaoudov et al., 1995). It starts to increase at day 13 and reaches 3699 ng/ml at day 28, a five fold increase (Table 1). There is a simultaneous two-fold increase in plasma benzoylecgonine between day 6 and 28. The plasma concentrations of both cocaine and benzoylecgonine correlate with the duration of cocaine exposure ($r = 0.94$ and $r = 0.79$, $P < 0.001$, respectively).

3.2. Anatomical data. Hemodynamic measurements

Cocaine administration was well-tolerated and the body weight remained unchanged (Table 2). All the animals continued to gain weight during the experimental procedure and survived throughout the study. The mean arterial blood pressure remained unaltered after chronic cocaine administrations (103 ± 2 and 112 ± 30 mmHg after 14 days of treatment with saline and cocaine, respectively; 111 ± 2 and 108 ± 2 mmHg after 28 days) as well as heart rate (396 ± 6 and 413 ± 10 beats/min after 14 days of

Table 2
Anatomic parameters

	14 Days of treatment		28 Days of treatment	
	controls, $n = 12$	cocaine-treated, $n = 15$	controls, $n = 10$	cocaine-treated, $n = 10$
BW (g)	331 ± 6	340 ± 3	382 ± 8	389 ± 8
HW (mg)	939 ± 32	933 ± 21	1053 ± 25	1172 ± 60
LVW (mg)	679 ± 22	670 ± 18	751 ± 19	887 ± 49 [a]
RVW (mg)	197 ± 11	199 ± 7	225 ± 7	219 ± 10
HW/BW (mg/g)	2.83 ± 0.07	2.74 ± 0.06	2.75 ± 0.03	3.00 ± 0.09 [a]
LVW/BW (mg/g)	2.05 ± 0.05	1.97 ± 0.05	1.96 ± 0.03	2.26 ± 0.08 [c]
RVW/BW (mg/g)	0.59 ± 0.03	0.58 ± 0.02	0.59 ± 0.01	0.56 ± 0.02
LVW/RVW	3.53 ± 0.15	3.41 ± 0.14	3.36 ± 0.11	4.04 ± 0.10 [b]
Liver (mg/g BW)	42 ± 1	44 ± 1	40 ± 1	38 ± 1
Left kidney (mg/g BW)	3.56 ± 0.08	3.61 ± 0.06	3.27 ± 0.07	3.19 ± 0.07

BW indicates body weight; HW, heart weight; LVW, left ventricular weight; RVW, right ventricular weight. Values are mean ± S.E.M.
[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ vs. sham-operated control group.

treatment with saline and cocaine, respectively; 388 ± 8 and 407 ± 8 beats/min after 28 days). Fourteen days of cocaine administration had no effect on cardiac weight. A significant degree of left ventricular hypertrophy was observed after 28 days, the right ventricular weight did not change and the major anatomical finding was a 20% increase in the left to right ventricular weight ratio (Table 2).

3.3. Plasma hormones

The plasma concentrations of both free triiodothyronine (T3: 1.16 ± 0.08 vs. 1.42 ± 0.07 pg/ml in controls, $P < 0.05$) and thyroxine (T4: 14.8 ± 0.7 vs. 18.4 ± 0.46 pg/ml in controls, $P < 0.001$) were significantly decreased after 28 days of cocaine treatment. In contrast, the plasma concentrations in aldosterone (86 ± 14 vs. 91 ± 23 pg/ml in controls), cortisol (6.68 ± 0.47 vs. 5.51 ± 0.62 ng/ml in controls) and active renin (1.14 ± 0.41 vs. 0.57 ± 0.17 pg/ml in controls) were not significantly modified after this period of cocaine administration.

3.4. RNA determinations

The yield in total RNA was the same in the saline (in μg RNA per mg of fresh tissue: 0.73 ± 0.21, 0.51 ± 0.05, 0.78 ± 0.21, 0.61 ± 0.09, 0.87 ± 0.11 and 0.37 ± 0.06 after 30 min, 1, 2, 4 h, 14 and 28 days, respectively) and cocaine treated (0.46 ± 0.17, 0.52 ± 0.04, 0.55 ± 0.07, 0.57 ± 0.10, 1.13 ± 0.55 and 0.44 ± 0.08) groups. In addition, cocaine treatment did not modify the total mRNA content of the myocardium since the poly(A^+)/18S ratio remained unchanged after 14 days (0.70 ± 0.09 and 0.93 ± 0.04 in saline and cocaine-treated animals respectively, $p = 0.053$) and 28 days (0.23 ± 0.04 and 0.30 ± 0.07) of infusion.

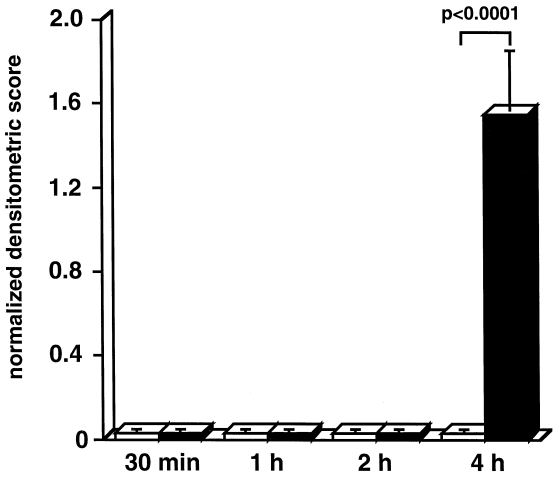

Fig. 2. Atrial natriuretic factor (ANF) gene expression in the left ventricle 30 min ($n = 7$ and 4, respectively), 1 h ($n = 7$ and 4, respectively), 2 h ($n = 7$ and 4, respectively) and 4 h ($n = 7$ and 7, respectively) after a single injection of cocaine (■) or saline solution (□).

In normal rat hearts, ANF is expressed in atria and not in ventricles. At all times and in all samples after acute injection of isotonic saline solution, ANF mRNA remained undetectable in the left ventricle as well as after 30 min, 1 h and 2 h of a single cocaine injection (Figs. 1 and 2). In contrast, ANF mRNA became detectable in all samples of left ventricles 4 h after a single injection of the drug (Figs. 1 and 2).

After 14 or 28 days of cocaine treatment, ventricular ANF expression remained elevated (Fig. 3). The myosin heavy chain mRNA pattern remained unchanged after 14 days of cocaine administration whatever the normalization procedure used (18S or poly (A^+)). In contrast, 28 days of cocaine administration induced a 42% decrease (in % of control values) in α-myosin heavy chain mRNA relative to

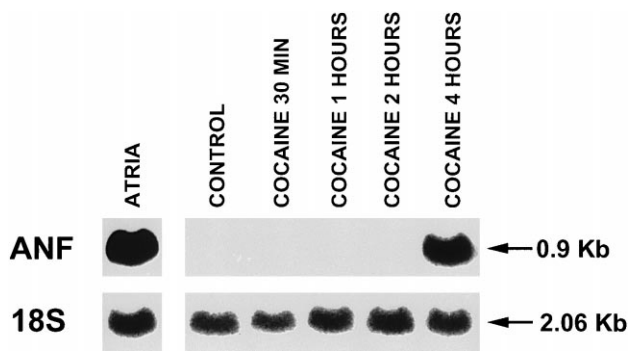

Fig. 1. Northern blot analysis showing the left ventricular expression of atrial natriuretic factor (ANF) in acutely-treated rats. 10 μg of total RNA from left atria (positive control for ANF) or 20 μg of total RNA from left ventricle of control and acutely cocaine-treated rats (30 min, 1, 2 and 4 h after injection of cocaine 40 mg/kg) were sequentially hybridized with ANF and 18S probes.

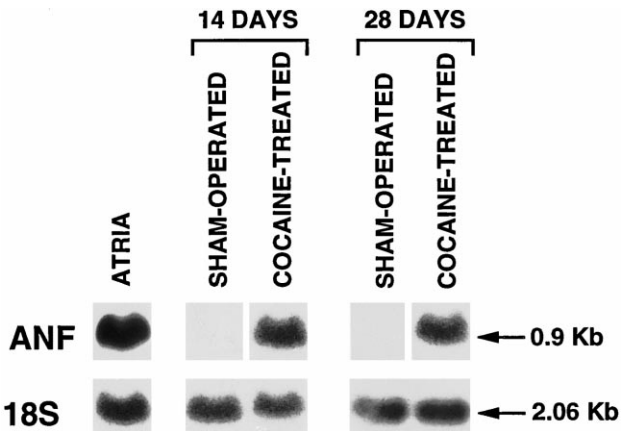

Fig. 3. Northern blot analysis showing the left ventricular expression of atrial natriuretic factor (ANF) during chronic infusion of cocaine (14 and 28 days). 10 μg of total RNA from left atria or 20 μg of total RNA from left ventricle of sham-operated controls or cocaine-treated rats were sequentially hybridized with ANF and 18S probes.

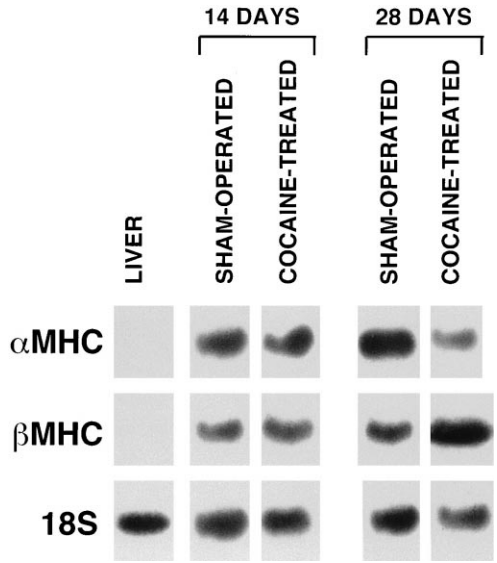

Fig. 4. Northern blot analysis showing the left ventricular expression of α- and β-myosin heavy chain (MHC) during chronic infusion of cocaine (14 and 28 days). 20 μg of total RNA from liver (negative control for α- and β-MHC) or 20 μg of total RNA from left ventricle of sham-operated controls or cocaine-treated rats were sequentially hybridized with α- and β-MHC and 18S probes.

the 18S content and a 104% increase in β-myosin heavy chain mRNA which matched the degree of left ventricular hypertrophy (Fig. 4 and Tables 2 and 3). Same results were obtained when the results were normalized to Poly(A^+) containing RNA (−42% and +70% for α-myosin heavy chain and β-myosin heavy chain, respectively, Table 3). Both α1(I) (type I) and α1(III) (type III) procollagen mRNA levels were unchanged after 14 days, and increased after 28 days (+66% and +47% for the α1(I) and α1(III) procollagen mRNA/18S ratios, respectively) (Table 3). Such an increase remained significant when the mRNA levels were normalized for the poly(A^+) content (+78% and +57%, respectively) (Table 3).

4. Discussion

The main findings of this study are the followings. (1) The mRNAs encoding ANF in the left ventricle accumulate 4 h after a single cocaine injection and remain present after chronic cocaine treatments. (2) 28 days of cocaine infusion result in left ventricular hypertrophy associated with a shift in myosin heavy chain mRNAs from α to β isoform and an accumulation of both type I and III procollagen transcripts.

The first report concerning changes in genetic expression during cocaine administration was in rat brain (Hope et al., 1992). Acute and chronic cocaine treatment induces the activation of several early genes which could regulate genes implicated in brain function (Hope et al., 1992). In myocardium, acute cocaine administration induces ANF gene expression 4 h after a single injection. In rats, acute injection of cocaine results in a well-documented immediate dose-dependent rise in blood pressure (Hollander, 1995; Pelkonen et al., 1996) associated with a dose dependent increase in plasma levels of ANF (Pelkonen et al., 1996). Such an increase in blood pressure which mediates ANF release (Pelkonen et al., 1996) is also probably responsible for the burst in ventricular ANF gene expression observed 4 h after a single cocaine injection.

Both 14 and 28 days of cocaine administration are also associated with ANF mRNA accumulation in spite of normal blood pressure. Indeed, blood pressure returns to control levels 5 days after the beginning of a chronic cocaine infusion (Johansson et al., 1992) and is similar to control levels after 14 and 28 days of cocaine administration. In long term cocaine abusers, clinical findings indicate that the systolic blood pressure is normal (Brickner et al., 1991). Therefore, the sustained increase of ANF mRNAs observed during chronic cocaine exposure and the associated hypertrophy may be related to other causes than pressure overload.

Table 3
Myosin heavy chain and collagen mRNA levels in left ventricles from both 14 and 28 days-treated rats

	14 Days treatment		28 Days treatment	
	controls, $n = 12$	cocaine-treated, $n = 15$	controls, $n = 10$	cocaine-treated, $n = 10$
α MHC/Poly(A^+)	1.00 ± 0.07	0.98 ± 0.12	1.06 ± 0.18	0.61 ± 0.10 [a]
α MHC/18S	0.90 ± 0.07	0.84 ± 0.13	0.25 ± 0.03	0.15 ± 0.03 [a]
β MHC/Poly(A^+)	1.50 ± 0.19	2.30 ± 0.35	4.03 ± 0.63	6.80 ± 0.82 [a]
β MHC/18S	1.23 ± 0.14	1.42 ± 0.34	0.98 ± 0.22	1.99 ± 0.38 [a]
Collagen type I/Poly(A^+)	0.97 ± 0.08	1.60 ± 0.33	1.03 ± 0.11	1.83 ± 0.28 [a]
Collagen type I/18S	0.88 ± 0.09	0.69 ± 0.07	0.27 ± 0.03	0.45 ± 0.07 [a]
Collagen type III/Poly(A^+)	0.94 ± 0.16	1.36 ± 0.28	0.67 ± 0.09	1.03 ± 0.13 [a]
Collagen type I/18S	0.82 ± 0.12	0.75 ± 0.14	0.16 ± 0.02	0.24 ± 0.03 [a]

Myosin heavy chain (MHC) and collagen mRNA levels were normalized by poly(A^+) or 18S RNA and expressed in normalized densitometric scores. Mean ± S.E.M.

[a] $P < 0.05$ vs. control group.

Twenty-eight days of cocaine administration is also associated with an alteration of the myosin heavy chain pattern of gene expression similar to that observed after cardiac overload (Lompré et al., 1979; Feldman et al., 1993; Robert et al., 1994), i.e., a shift from α- to β-myosin heavy chain gene expression. The β-myosin heavy chain isogene encodes for a myosin subunit with slow ATPase activity and such an isomyosin shift correlates with alterations of the maximum unloaded shortening velocity (V_{max}) (Apstein et al., 1987; Besse et al., 1993). This isomyosin shift may be attributed, at least in part, to hypothyroidism which also induces a reexpression of the β-myosin heavy chain (Izumo et al., 1987) since chronic cocaine intoxication is associated with low levels of plasma triiodothyronine and thyroxine. However, the additional existence of sustained increase of ANF mRNAs as well as left ventricular hypertrophy during chronic cocaine exposure rather suggests a myocyte overload by other causes such as ischemia.

The alterations in contractile proteins gene expression are associated with left ventricular hypertrophy in rats after 28 days of intoxication. Such a left ventricular hypertrophy was found in humans after several years of cocaine abuse (Virmani et al., 1988; Brickner et al., 1991; Cigarroa et al., 1992) and could play a potentiating role in the previously observed ischemic and arrhythmogenic properties of cocaine (Hollander, 1995). This study provides some insights on the mechanism by which cocaine abuse causes left ventricular hypertrophy. After 28 days of cocaine treatment, plasma cocaine concentration has increased 5 fold over its levels at day 6, which might reflect a saturation of its metabolizing enzymes, and probably results in tissue accumulation. At this time, both ANF and myosin heavy chain mRNA changes indicate a sustained myocyte overload associated with a left ventricular hypertrophy which cannot be related to arterial hypertension, as previously observed in chronic cocaine abusers (Brickner et al., 1991). Such a chronic myocyte overload could, at least partially, be attributed to myocardial ischemia. Myocyte necrosis associated with 'contraction bands', followed by loss and degeneration of myofibrils, as well as non-muscular cells proliferation such as macrophages, lymphocytes and fibroblasts have indeed been previously reported in the same model (Maillet et al., 1991). Similar lesions have also been reported in man (Karch and Billingham, 1988; Virmani et al., 1988). In addition, 28 days of chronic cocaine exposure induce a strong activation of genes encoding type I and III collagens suggesting an active process of incipient reparative fibrosis.

Cocaine abuse can provoke myocardial injury and necrosis by (1) coronary vasoconstrictive effect (Lange et al., 1989; Zimmring et al., 1994), but the fact that local ischemic areas were both interstitial and perivascular (Virmani et al., 1988; Maillet et al., 1991) argues against this possibility, (2) direct cardiotoxic effects (Stewart et al., 1991; Qiu and Morgan, 1993) which are not mediated by a release of catecholamines, (3) alteration of calcium homeostasis (Tomita et al., 1993; Yuan and Acosta, 1994) leading to cytosolic calcium overload, (4) an increase in circulating catecholamines (Pitts et al., 1987), which is known to cause areas of necrosis and to induce both the expression of collagen genes and left ventricular hypertrophy (Bhambi and Eghbali, 1991).

In conclusion, chronic cocaine intoxication induces changes in the expression of genes encoding myosin heavy chain and collagens together with both sustained expression of ANF and left ventricular hypertrophy, indicating a cardiac overload. The direct and/or indirect mechanisms of this cocaine-induced left ventricular hypertrophy, which is not related to increased blood pressure, remain to be determined. Such changes in contractile proteins and extracellular matrix may result in myocardial impairment and damage, previously reported in chronic cocaine abusers.

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