ELSEVIER

European Journal of Pharmacology 398 (2000) 263–272

ejp

www.elsevier.nl/locate/ejphar

Combined cardiac effects of cocaine and the anabolic steroid, nandrolone, in the rat

Benjamin D. Phillis [a], Rodney J. Irvine [a], Jennifer A. Kennedy [b,*]

[a] *Department of Clinical and Experimental Pharmacology, University of Adelaide, Frome Road, Adelaide 5000, Australia*
[b] *Cardiology Unit, The Queen Elizabeth Hospital, Woodville 5011, Australia*

Received 4 October 1999; received in revised form 31 March 2000; accepted 7 April 2000

Abstract

Despite reports of an increase in the incidence of simultaneous cocaine and anabolic steroid abuse, potential adverse interactions between these two drugs on the cardiovascular system are largely unquantified. Cocaine has been reported to induce coronary vasoconstriction, cardiac arrhythmias and conduction delays. Anabolic steroids have been associated with cardiac hypertrophy and hypertension. Utilising both in vivo (radiotelemetry) and in vitro (isolated Langendorff-perfused heart) techniques, our aim was to determine whether anabolic steroids cause cardiac hypertrophy and alter cardiac function, and consequently alter the response of the heart to cocaine. It was found that 15 days of treatment of rats with nandrolone decanoate (20 mg/kg, s.c.) was not sufficient to cause hypertrophy, alter cardiac function or the spread of electrical activity through the heart. However, nandrolone pretreatment was found to significantly potentiate the heart rate response to cocaine (45 mg/kg, i.p.) in vivo. This study indicates that nandrolone significantly elevates the heart rate response to high dose cocaine without changing heart morphology. The mechanism of this interaction remains uncertain.

Keywords: Nandrolone decanoate; Deca durabolin; Cocaine; Radiotelemetry; Cardiac hypertrophy; ECG (electrocardiogram); Noradrenaline

1. Introduction

Polydrug abuse is not a recent phenomenon, therefore, it is surprising that very few studies have examined the toxic effects of commonly used drug combinations. Epidemiological reports suggest that both cocaine and anabolic steroids alone are a significant abuse problem. For example, it has been estimated that there were 1.4 million cocaine users in the United States in 1994 (cited Cornish and O'Brien, 1996), while data from a 1991 survey indicated that there were more than one million current or former anabolic steroid users in the United States (Yesalis et al., 1993). Recent pharmacoepidemiological studies have found that the frequency of anabolic steroid use is associated with the frequency of cocaine abuse (DuRant et al., 1993, 1995). Despite this evidence of a potentially significant number of patients co-abusing anabolic steroids and cocaine, the possible cardiac damage induced by this combination remains unknown.

Both anabolic steroid and cocaine have been associated with acute and chronic cardiac effects. Acute administration of cocaine has been associated with a range of adverse cardiac effects including coronary vasoconstriction (Vitullo et al., 1989; Chokshi et al., 1990; Lange et al., 1989), arrhythmias (Schwartz et al., 1989; Temesy-Armos et al., 1992), conduction delays and re-entrant circuits (Billman and Lappi 1993; Kabas et al., 1990; Schwartz et al., 1989). Transient coronary vasoconstriction has been postulated to give rise to an imbalance in oxygen supply and demand and increased risk of cardiac ischaemia and myocardial infarction (Lange et al., 1989). Cardiac arrhythmias are thought to arise from increased catecholamine concentrations resulting in activation of α and β adrenoceptors and increased intracellular calcium (Billman, 1990, 1995). Conduction delays and re-entrant circuits have been attributed to the local anaesthetic action of cocaine (Billman and Lappi, 1993). Chronic abuse of cocaine has been linked to cardiac hypertrophy (Karch et al., 1995; Brickner et al., 1991), while chronic treatment of rats with cocaine has been shown to produce left ventricular hypertrophy (Besse et al., 1997). Unlike cocaine, the acute cardiac

* Corresponding author. Tel.: +61-8-82226679; fax: +61-8-82227181.
E-mail address: jenny.kennedy@nwahs.sa.gov.au (J.A. Kennedy).

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00294-6

effects of anabolic steroid are largely unquantified. A number of authors have suggested that chronic anabolic steroid use may contribute to cardiac hypertrophy (McKillop et al., 1986; Tseng et al., 1994; Kennedy and Lawrence, 1993; Luke et al., 1990). More recently, a direct hypertrophic effect of testosterone and dihydrotestosterone has been demonstrated in isolated cardiomyocytes (Marsh et al., 1998).

Cocaine is thought to have a dual mode of action. At low doses, cocaine has been found to induce a sympathomimetic effect mediated through prevention of the reuptake of noradrenaline. At higher doses, cocaine is thought to have a local anaesthetic effect by changing the permeability of axon membranes to sodium ions (Shakalis and Condouris, 1967; Pitts and Marwah, 1989) and blocking calcium-dependent potassium channels (Grossie, 1993).

Using indirect measurements of blood pressure and heart rate in developing male spontaneously hypertensive rats, Tseng et al. (1994) demonstrated that treatment for 6 weeks with nandrolone or cocaine, or a combination of both drugs, accelerated the development of hypertension. A combination of nandrolone and cocaine produced a greater degree of cardiac hypertrophy than either drug alone, while only the combined drug treatment produced a significant decrease in heart rate.

Since no information is available on the combined effect of these drugs in adult animals, the purpose of the present study was to determine whether an interaction between cocaine and nandrolone treatment on cardiac function occurs in the adult normotensive male rat. It was hypothesised that steroid treatment would cause cardiac hypertrophy, changes in cardiac function and alterations in the cardiac response to cocaine. Cocaine was administered intraperitoneally (i.p.) to the rats since its disposition pattern by this route closely resembles that following intranasal administration in the human (Javaid and Davis, 1993). Radiotelemetric monitoring of electrocardiogram (ECG) was used to provide a direct measure of heart rate and conduction through the heart without the complication of stress-induced changes in cardiac function which accompany the handling and restraint (Irvine et al., 1997) involved in indirect measures of blood pressure and heart rate. In addition, the in vivo studies (radiotelemetry) were complimented by in vitro studies using the Langendorff-perfused heart preparation to assess possible cardiac damage resulting from steroid pretreatment, and any potential modification of the direct toxic effects of cocaine on the heart caused by the pretreatment with nandrolone.

2. Materials and methods

2.1. Animals

Adult male albino Wistar rats were purchased from Central Animal Supplies (Waite Campus, University of Adelaide). Rats were provided with standard rat chow and water ad libitum. Following surgery, rats were housed individually and were maintained in a standard 12-h light–dark cycle, beginning at 7 AM. Rats were subjected to a constant room temperature of 22 ± 2°C. Animals were randomly assigned to either of the two treatment groups.

2.2. Radiotelemetry

Radiotelemetry implants (Physiotel implant, model TA11CTA-F40, Data Sciences, St. Paul, MN) allowed the measurement of heart rate, temperature, spontaneous locomotor activity and ECG in freely moving, fully conscious rats. Each implant (approximately 30×17 mm) had two electrodes, silastic coated, with approximately 10 mm of exposed wire near the end of the electrodes. The tips of each electrode were covered to prevent local irritation developing. Signals from the implants were collected by a receiver (RA1020 Receiver, Data Sciences), which was connected to 486 computer running LabPro software (Data Sciences). Each implant was calibrated for temperature and ECG to conform to the manufacturers configuration settings. The waveform sampling rate was set at 1000 Hz with a 250-Hz filter.

2.3. Surgical procedure

Adult male albino Wistar rats weighing 509 ± 20 g were anaesthetised with a mixture of methohexitone sodium (10 mg/ml) and pentobarbital sodium (60 mg/ml), administered i.p. in the ratio 10:1 at 5 ml/kg. Radiotelemetry implants were placed in the peritoneal cavity and stitched to the abdominal wall with non-absorbable suture. The positive electrode was secured subcutaneously just left of the xyphoid process. The negative electrode was tunnelled under the skin to the area of the right scapula and stitched in place. Both electrodes were immobilised with non-absorbable suture to prevent migration and to reduce movement interference. Wound sites were irrigated with bupivacaine 0.5% to reduce post-operative pain. Systemic and topical antibiotic was administered post-operatively. Rats were allowed to recover for a minimum of 10 days before experimentation was begun. Full details of the implants and surgical procedure are described elsewhere (Brockway et al., 1991).

2.4. Treatments

Rats were treated with nandrolone decanoate (20 mg/kg, s.c.) or vehicle (peanut oil supplemented with 10% benzyl alcohol) for 17 days, three mornings per week (Monday, Wednesday, Friday). The final nandrolone and peanut oil injections were given on the Wednesday of the third week. In the third week of treatment, rats were

administered increasing concentrations of cocaine HCl (5, 15 and 45 mg/kg, i.p.) on three consecutive afternoons (Monday, Tuesday, Wednesday). Saline was administered late Monday morning (i.p.) prior to the administration of 5 mg/kg cocaine.

2.5. Langendorff-perfused hearts

Isolated hearts were perfused retrogradely via the aorta in the Langendorff mode. The heart was perfused at a constant pressure of 60 mm Hg with Krebs bicarbonate solution at 37°C and gassed with 95% O_2/5% CO_2. Krebs solution was made with the following composition: sodium chloride (118 mM), potassium chloride (4.7 mM), sodium hydrogen carbonate (25 mM), D-glucose (5.6 mM), potassium dihydrogen orthophosphate (1.2 mM), calcium chloride (2.5 mM), magnesium chloride (0.74 mM), EDTA (0.012 mM). Krebs solution was filtered through a 1.2-μm millipore filter before use. All Krebs components were analytical grade purity. Changes in coronary flow rate through the perfused heart were detected using an electromagnetic flow probe attached to a dual channel flowmeter (Zepeda Instruments, Seattle, WA, USA). Changes in left ventricular pressure and heart rate of the isolated heart, were detected using a pressure transducer (Statham P23XL) attached to a latex balloon on a stainless steel catheter. The balloon catheter was placed in the left ventricle of the heart via the pulmonary vein. Changes in heart rate, left ventricular pressure and coronary flow rate were continuously monitored using an eight-channel MacLab Data Collation Unit (MacLab/8e, AD Instruments, Castle Hill, NSW). Data was collected using Chart v 1.1 from MacLab (AD Instruments). At the conclusion of the perfusion period, the hearts were blotted dry, and the intraventricular fluid was gently squeezed out. Heart weight to body weight ratio (day 15 of treatment) was calculated.

2.6. Experimental protocol

2.6.1. In vivo: radiotelemetry recording: pretreatment period

Following administration of nandrolone or vehicle, individually housed rats were placed in their home cage on top of the telemetry receivers and allowed 60 min to recover from the stress of injection. Heart rate, temperature, locomotor activity and ECG were subsequently measured for 60 min. Recordings of these variables were taken once every 10 min for 10 s.

2.6.2. In vivo: radiotelemetry recording: cocaine administration

On day 15 of pretreatment, cocaine administration was begun. Radiotelemetry recording was conducted immediately following cocaine injection and continued for 80 min post injection. The maximum elapsed time between cocaine administration and the start of recording was 2 min. Recording of heart rate, temperature, locomotor activity and ECG was conducted every 5 min for 10 s.

2.6.3. In vitro: Langendorff-perfused hearts

Following completion of the in vivo protocol, rats were sacrificed and the hearts excised into chilled, calcium-free, Krebs solution. This prevented the hearts from beating, until they were attached to the aortic cannula, and perfusion with Krebs solution had begun. With the balloon deflated, the catheter was pushed through the pulmonary vein of the rat heart into the left ventricle. Once the balloon was in the ventricle, it was inflated with water from a spindle syringe and the catheter tied in place. Diastolic pressure was set at 5 mm Hg. Hearts were equilibrated for 20 min before cocaine (0.1–100 μM) was added cumulatively to the perfusing Krebs solution and the left ventricular pressure, coronary flow rate and heart rate measured at each concentration of cocaine. To determine whether there was any contribution from spontaneously released noradrenaline in the presence of cocaine, this same dose–response relationship to cocaine was then determined in the presence of α and β adrenoceptor blockade, with phentolamine (1 μM) and propranolol (1 μM), respectively.

2.7. Data analysis

2.7.1. Radiotelemetry

Results are expressed as means ± S.E. The minimum level for statistical significance was $P < 0.05$.

Changes in heart rate, temperature, locomotor activity over time were assessed using repeated-measures analysis of variance (ANOVA) with unpaired *t*-test post hoc. The peak effect and area under the curve (AUC) for heart rate, temperature and locomotor activity in response to saline and cocaine (5–45 mg/kg, i.p.) for either pretreatment was determined. AUC was calculated using the trapezoidal method. Between- and within-pretreatment group changes in peak measures and AUC were assessed using an unpaired two-tailed *t*-test, or a single factor ANOVA with Tukey's multiple comparison test (respectively).

An average or representative waveform for each 10-s recording period was computed. From this wave, the PR, QRS and QT intervals (in ms) were measured. The QTc interval was derived from the QT interval. The QTc interval represents the QT interval adjusted for heart rate and was calculated using the formula...

$$QTc = \frac{QT}{\sqrt{RR}}$$

where RR is the RR interval (time in milliseconds between successive R peaks).

ECG data were presented by determining the interval duration at 10, 20, 30 and 40 min post saline or cocaine injection (45 mg/kg, i.p.). Significant changes between pretreatment groups were tested using an unpaired two-tailed *t*-test. Significant differences in the ECG within-pretreatment groups between saline and 45 mg/kg cocaine were determined using a paired, two-tailed *t*-test. Continuous trace ECGs were analysed for each pretreatment group at the highest dose of cocaine used (45 mg/kg, i.p.). The incidence or absence of arrhythmia was noted.

2.7.2. Langendorff-perfused hearts

Data were analysed using Chart v1.1 from MacLab (AD Instruments). The average of coronary flow rate, heart rate and left ventricular pressure were determined over a 60-s period at the peak of the drug effect. Left ventricular developed pressure (i.e. pulse height) was calculated from the difference between left ventricular systolic pressure and diastolic pressure. Rate pressure product was used as a measure of systolic function and was calculated from the product of left ventricular developed pressure and heart rate. The coronary flow rate, heart rate and rate pressure product were expressed as percent of baseline (pre-drug) values for increasing cocaine concentrations. These data were presented as a logarithmic concentration response curve. Differences in coronary flow rate, heart rate and rate pressure product due to pretreatment, prior to the addition of cocaine, were assessed by unpaired *t*-test. Overall changes in coronary flow rate, heart rate and rate pressure product in response to cocaine between and within pretreatment groups were calculated using repeated-measures ANOVA and an unpaired *t*-test for selected between-pretreatment group differences.

2.8. Drugs

Methohexitone sodium (Brietal) was obtained from Eli Lilly (Indianapolis, USA), pentobarbital sodium (Nembutal) from Boehringer Ingelheim (Artarmon, NSW, Australia) and bupivacaine 0.5% (Marcain) from Astra (North Ryde, NSW, Australia). A stock solution of methohexitone sodium was made up to 10 mg/ml in 0.9% sterile saline. Antibiotics used during surgery included TRIBRISSEN (Trimethoprim 80 mg/ml, Sulfadiazine 400 mg/ml) from Jurox (Silverwater, NSW, Australia) and topical antibiotic powder (neomycin sulphate 2.5 mg/g, sulphacetamide sodium 100 mg/g, nitrofurazone 2 mg/g, benzocaine 5 mg/g) from Apex (St. Mary's, NSW, Australia). Nandrolone decanoate (DECA-DURABOLIN) was donated by Organon (Sydney, NSW, Australia). Cocaine HCl was obtained from Delta-West pharmaceuticals (Perth, WA, Australia). Vehicle (arachis oil) was obtained from F.H. Faulding (Salisbury, SA, Australia). Benzyl alcohol was purchased from Ajax Chemicals (Auburn, NSW, Australia). Vehicle was used as diluent to make a 10% solution of benzyl alcohol. Both propranolol and phentolamine were obtained from Sigma (St. Louis, MO, USA).

3. Results

3.1. Pretreatment period: in vivo

3.1.1. Body weight changes

All groups tolerated the chronic treatment regime well showing either no change or increased body weight over the pretreatment period (days 1–15). The changes were 0 ± 4 and 17 ± 5 g for nandrolone and vehicle treatment groups, respectively. The weight gain in vehicle treated rats was significantly greater than in the nandrolone-treated group ($P < 0.05$, unpaired *t*-test). Thus, in unexercised, adult male animals, there was no evidence of a generalised anabolic effect of nandrolone.

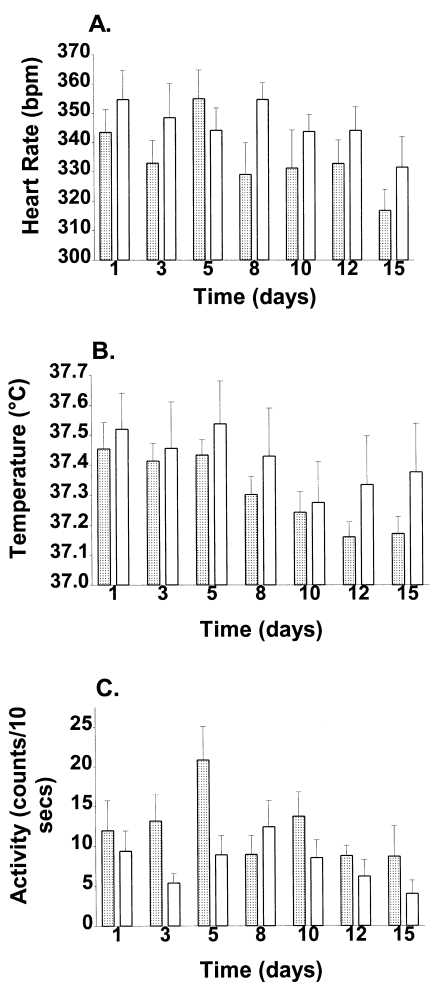

Fig. 1. Mean (A) heart rate, (B) temperature, (C) locomotor activity in AW rats treated with nandrolone (20 mg/kg, s.c.) (shaded bars), or vehicle (open bars) for 15 days. ±S.E., $n = 6–9$. Repeated-measures ANOVA, unpaired *t*-test.

3.1.2. Heart weight

In contrast to expectations there was no evidence of cardiac hypertrophy in response to nandrolone compared with controls. Percent heart weight/body weight ratios were $0.332 \pm 0.011\%$ and $0.343 \pm 0.013\%$ for nandrolone and vehicle pretreatment groups, respectively. No significant difference in the heart weight/body weight ratio was found.

3.1.3. Heart rate, temperature and locomotor activity changes

Radiotelemetry recording detected no statistically significant changes in heart rate, temperature and spontaneous locomotor activity between the two pretreatment groups over the duration of the pretreatment period (Fig. 1). It would appear that nandrolone treatment did not influence the functional physiology as assessed by these measures.

3.1.4. ECG changes

ECG measurements were in keeping with the lack of change in heart weight. Radiotelemetry recording detected no significant difference in the magnitude of the PR, QRS, QT and QTc intervals over the duration of the pretreatment period between the treatment groups.

3.1.5. Pretreatment period: in vitro

The data collected from perfused isolated hearts was consistent with that recorded in vivo. No significant differences in basal coronary flow rate, heart rate or rate pressure product in isolated hearts, from nandrolone- and vehicle-pretreated rats were evident. Overall, treatment with nandrolone did not have a great influence on the animal's gross morphology, behaviour or physiology as measured by the parameters assessed.

3.1.6. Cocaine administration

Heart rate, temperature and locomotor activity all displayed dose-dependent increases in magnitude in response to increasing cocaine dose both in terms of AUC and peak effect for each of these parameters. As AUC and peak effect both demonstrate a common dose-reliant trend, only the results for AUC will be presented in detail.

3.1.7. Heart rate response to cocaine.

Increasing cocaine dose caused an incremental increase in heart rate in both pretreatment groups (Figs. 2 and 3). Significant differences between the heart rate response to cocaine for either pretreatment were found at individual time points following drug administration (Fig. 2).

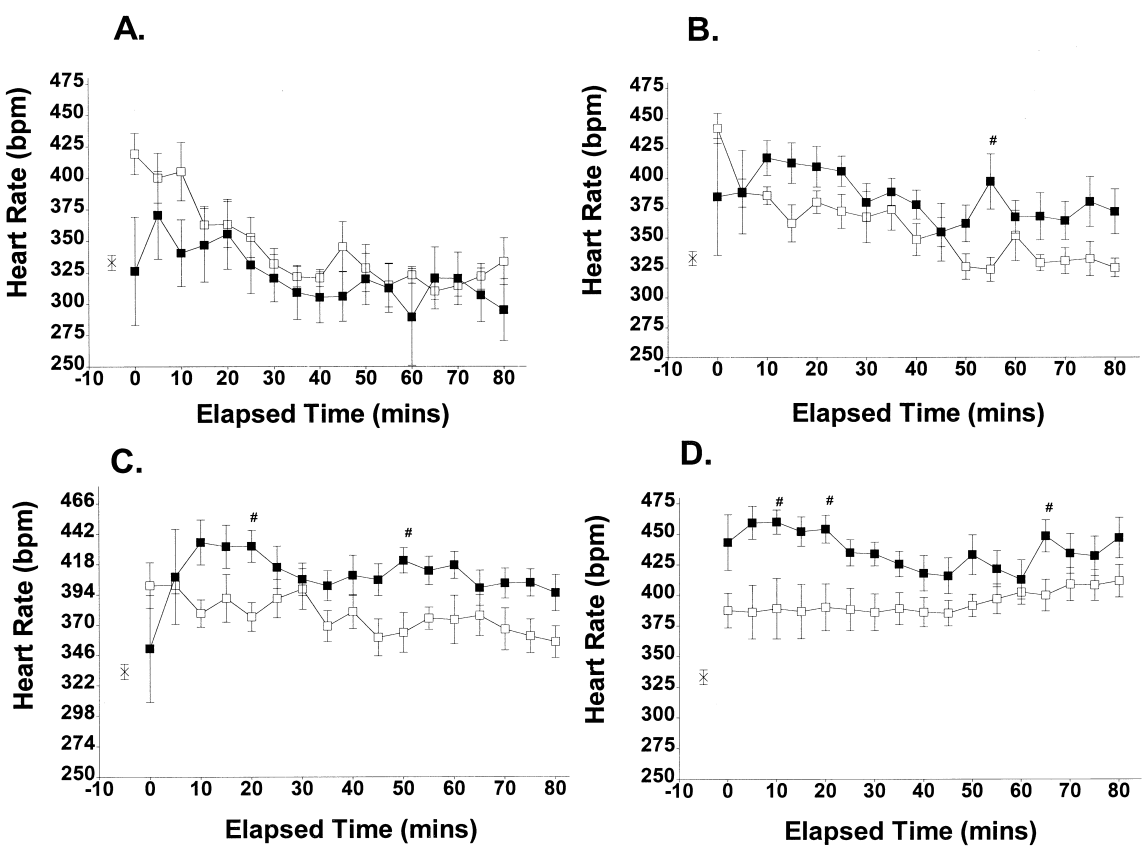

Fig. 2. Heart rate changes in AW rats pretreated with nandrolone (20 mg/kg, s.c.) (■) or vehicle (□) and subsequently administered saline or cocaine; (A) saline, i.p., (B) cocaine 5 mg/kg, i.p., (C) cocaine 15 mg/kg, i.p., (D) cocaine 45 mg/kg, i.p., mean ± S.E., $n = 6–9$. $\#P < 0.05$, significantly different from vehicle group. (X) Represents mean resting heart rate (b.p.m.) for all groups before treatment. Repeated-measures ANOVA, unpaired *t*-test.

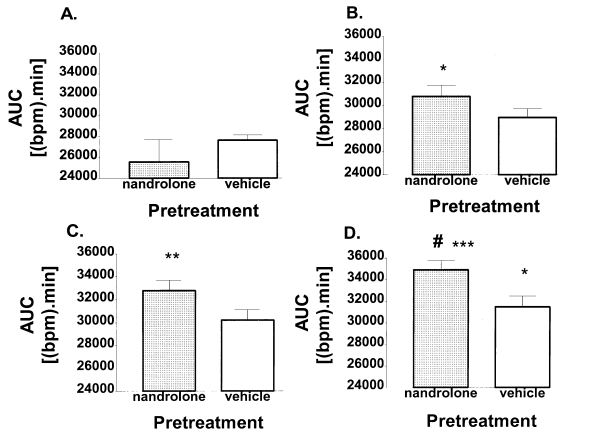

Fig. 3. AUC ± S.E. for heart rate vs. time graph in AW rats pretreated with nandrolone 20 mg/kg, s.c. (shaded bars) or vehicle (open bars) and administered (A) saline, or cocaine (B) 5 mg/kg, i.p., (C) 15 mg/kg, i.p., (D) 45 mg/kg, i.p., mean ± S.E., $n = 6–9$. $\#P < 0.05$, nandrolone-pretreated group significantly different from vehicle-pretreated groups, unpaired *t*-test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, i.p. cocaine significantly different from i.p. saline, single-factor ANOVA, unpaired *t*-test.

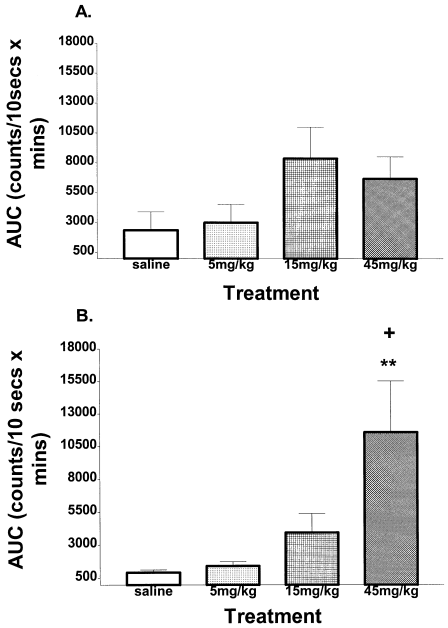

Fig. 4. Total area under the locomotor activity vs. time graph in AW rats pretreated with (A) nandrolone (20 mg/kg, s.c.), (B) vehicle and administered saline or cocaine (5–45 mg/kg, i.p.), mean ± S.E., $n = 6–9$. $^{**}P < 0.1$, significantly different from saline; $+ P < 0.05$, significantly different from 15 mg/kg. Single-factor ANOVA, Tukey's multiple comparisons test.

Nandrolone-pretreated rats had a significantly greater ($P < 0.05$) mean AUC for heart rate vs. time when compared to control with 45 mg/kg cocaine (Fig. 3D). Likewise, the peak heart rate response to cocaine (5–45 mg/kg) was always highest in nandrolone-pretreated rats. This difference in nandrolone-pretreated rats was only statistically greater than in control for 45 mg/kg cocaine ($P < 0.01$).

3.1.8. Temperature response to cocaine

Core temperature did not significantly increase with the administration of cocaine in both nandrolone-pretreated and control rats (Table 1).

3.1.9. Locomotor activity response to cocaine

A dose-dependent increase in locomotor activity was observed in both pretreatment groups (Fig. 4). The effects of cocaine on locomotor activity were not influenced by the pretreatment (e.g. $P = 0.27$ for nandrolone-pretreated and vehicle-pretreated groups with respect to response to 45 mg/kg cocaine, unpaired *t*-test).

Table 1
Body temperature response to cocaine
Body temperature (°C) response to cocaine (5–45 mg/kg) in AW rats pretreated with nandrolone or vehicle. Mean ± S.E., $n = 6–9$, repeat measure ANOVA, unpaired *t*-test.

Cocaine (mg/kg, i.p.)	Nandrolone	Vehicle
0 (saline)	37.4 ± 0.2	37.3 ± 0.2
5	37.5 ± 0.2	37.5 ± 0.2
15	38.0 ± 0.3	37.8 ± 0.2
45	38.0 ± 0.3	37.7 ± 0.3

3.1.10. ECG

Analysis of continuous ECG traces from rats administered 45 mg/kg cocaine displayed no evidence of ectopic beats or arrhythmias in either pretreatment group. The PR interval was unaffected by saline or cocaine (45 mg/kg) injection and did not differ between pretreatment groups (Table 2). Injection of 45 mg/kg cocaine significantly increased QT interval at 10 and 40 min post injection in the nandrolone-pretreated rats and at 40 min post injection in vehicle-pretreated rats (Table 2). The unchanged or increased QT interval, combined with increased heart rate following 45 mg/kg cocaine, is reflected in the increased QTc interval, which was significant at most time points following injection of cocaine (Table 2). This did not appear to involve prolongation of the QRS interval since QRS remained constant within pre-treatment groups (e.g. at 40 min post i.p. injection, QRS (in ms) was 17 ± 1 following saline and 17 ± 1 following cocaine in vehicle-pretreated animals, and 17 ± 1 following saline and 16 ± 1 following cocaine in nandrolone-pretreated animals). There were no significant differences in QT or QTc between nandrolone and vehicle-treated rats following saline injection. However, cocaine-induced increases in QTc were significantly greater in the nandrolone-pretreated rats at two time points (30 and 40 min) after cocaine injection (Table 2), while the QT interval was greater in the nandrolone pretreated rats only at 30 min after injection.

Table 2
Effect of drug pretreatment on changes in ECG parameters in response to i.p. cocaine
Changes in PR, QT and QTc intervals in AW rats pretreated with nandrolone or vehicle and administered saline or cocaine (45 mg/kg, i.p.).

Pretreatment	Time (min)	PR (ms)		QT (ms)		QTc (ms)	
		Saline	Cocaine, 45 mg/kg	Saline	Cocaine, 45 mg/kg	Saline	Cocaine, 45 mg/kg
Nandrolone	10	47 ± 1	49 ± 2	66 ± 1	74 ± 1*	165 ± 4	205 ± 4**
	20	51 ± 1	53 ± 2	71 ± 3	69 ± 3	165 ± 8	197 ± 6**
	30	49 ± 1	46 ± 1	69 ± 1	73 ± 1#	163 ± 4	196 ± 3**,##
	40	49 ± 1	46 ± 1	66 ± 2	73 ± 1*	153 ± 6	190 ± 6**,#
Peanut oil	10	49 ± 1	52 ± 2	67 ± 3	71 ± 3	174 ± 12	179 ± 11
	20	51 ± 2	51 ± 2	61 ± 4	70 ± 2	148 ± 9	178 ± 8**
	30	49 ± 1	48 ± 1	63 ± 3	65 ± 3	150 ± 7	156 ± 10
	40	50 ± 1	48 ± 1	61 ± 3	67 ± 3*	142 ± 6	169 ± 7**

* Within-pretreatment effects: $P < 0.05$, significantly different from saline (paired t-test).
** Within-pretreatment effects: $P < 0.01$, significantly different from saline (paired t-test).
# Between-pretreatment groups: $P < 0.05$, significantly different from vehicle (unpaired t-test, $n = 6–9$).
Between-pretreatment groups: $P < 0.01$, significantly different from vehicle (unpaired t-test, $n = 6–9$).

Hence, the effect of nandrolone pretreatment on the QTc response to i.p. cocaine was not evident at all times after cocaine injection. Differences between pretreatment groups in QTc were not due to differences in QRS duration (e.g. at 30 min post cocaine injection, QRS in milliseconds: vehicle pretreatment 17 ± 1; nandrolone pretreatment 17 ± 1).

3.1.11. In vitro Langendorff-perfused hearts

Coronary flow rate, heart rate and rate pressure product all decreased with increasing cocaine dose (Fig. 5A,B,C, respectively). Propranolol (1 μM) and phentolamine (1 μM) had no significant effect on the dose response to cocaine (graph not shown) indicating that the effect of cocaine was not mediated via α or β adrenoceptors.

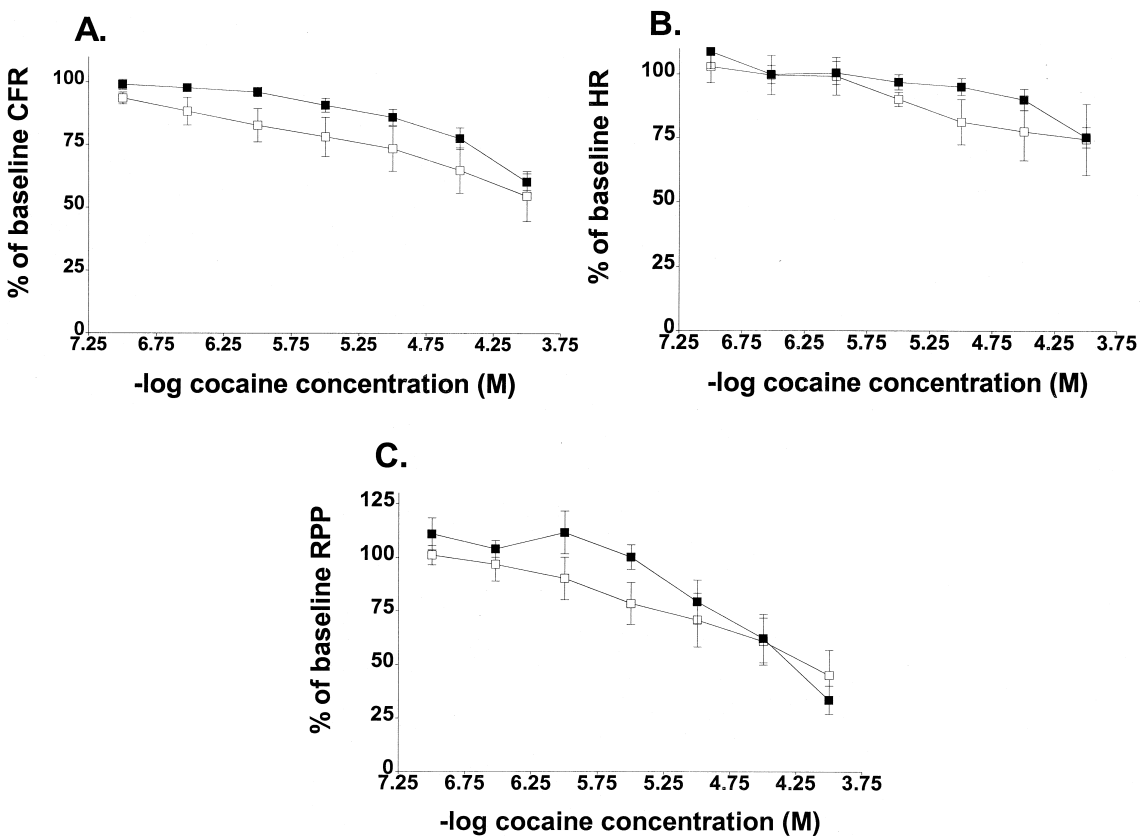

Fig. 5. Concentration–effect relationship for cocaine on (A) coronary flow rate, (B) heart rate, (C) rate pressure product (left ventricular developed pressure × heart rate) in response to cocaine (0.1–100 μM) in isolated hearts from AW rats pretreated with nandrolone (20 mg/kg, s.c.) (closed squares) or vehicle (open squares), mean ± S.E., $n = 6–9$. Repeated-measures ANOVA, unpaired t-test.

3.1.12. Between-group changes in coronary flow rate, heart rate and rate pressure product

No significant difference was found in the coronary flow rate, heart rate or rate pressure product response to cocaine between the two pretreatment groups (Fig. 5). Likewise, no significant difference was found in the coronary flow rate, heart rate or rate pressure product response to cocaine between the two pretreatment groups in the presence of propranolol and phentolamine.

3.1.13. Arrythmias

No arrhythmias were observed, which could be attributed to cocaine or to the pretreatment.

4. Discussion

Nandrolone administration did not result in cardiac hypertrophy in adult male rats in the present study. The increased heart weight to body weight ratio observed by Tseng et al. in developing spontaneously hypertensive rats may have resulted from hyperplastic changes to the cardiomyocytes (Kinson et al., 1991). Koenig et al. (1982) demonstrated that pre-pubertal rodents display changes in cardiac mass as a result of cardiac hyperplasia. However, it is possible that the shorter treatment period in the present study was insufficient to demonstrate a hypertrophic effect of nandrolone in adult rats.

Consistent with the observations of Tseng et al. (1994), nandrolone treatment for 15 days had no effect upon heart rate when compared to both of the control treatments. Likewise, no changes were observed in locomotor activity, temperature or any of the ECG intervals. The absence of effect on ECG suggests that pretreatment alone was not sufficient to alter cardiac function or the spread of electrical activity through the heart. This result was confirmed by the inability of nandrolone to alter the cardiac performance of the heart in vitro prior to the addition of cocaine. Furthermore, this study has demonstrated that nandrolone, in the absence of exercise did not increase body weight.

No adverse cardiac effects of nandrolone were observed in the isolated heart preparations since coronary flow rate, heart rate and rate pressure product were similar in all pretreatment groups. Cocaine did not cause arrhythmia in any pretreatment group. This suggests that cocaine did not act by a direct mechanism to alter cardiac rhythm. Nandrolone was not found to produce cardiac arrhythmias.

Direct cardiotoxic effects of high concentrations of cocaine were described by Simkhovitch et al. (1994) using the isolated rabbit heart, namely decreases in coronary flow rate, heart rate and contractility. Similar changes were observed in the present study on isolated rat hearts in which cocaine reduced coronary flow rate, heart rate and rate pressure product. These in vitro effects of cocaine were unaffected by α and β adrenoceptor blockade in either pretreatment group, indicating that endogenous noradrenaline release in the isolated hearts in the absence of sympathetic input is insufficient to contribute to an action of cocaine on the heart. These cardiotoxic effects of cocaine on isolated hearts were not influenced in any way by nandrolone pretreatment. Simkhovitch et al. (1994) demonstrated in paced rabbit hearts that decreases in cardiac contractility ($+dP/dt$) occurred earlier than decreases in heart rate or coronary flow rate. This suggests that the local anaesthetic action of cocaine may underlie its ability to cause depression of contractility, independent of heart rate and coronary flow rate. The cardiodepressant action of cocaine can be hypothetically linked to its action against sodium channels. High concentrations of cocaine have been found to produce a decrease in calcium release from the sarcoplasmic reticulum (SR) of ferret ventricular myocardium (Perreault et al., 1990). Because cocaine lowers the number of sodium ions entering the sarcoplasm via voltage-dependent sodium channels, this would lead to decreased sodium calcium exchange (Reiter, 1988). Consequently, this would result in lower amplitude calcium transients and negative inotropy. This proposed mechanism for the cardiodepressant effect of cocaine is supported by Stewart et al. (1991) who used isolated cardiomyocytes to demonstrate that 10 μM cocaine caused a 30% reduction in the release of calcium from cardiac cells.

In isolated hearts, cocaine was found to produce a profound and statistically significant decrease in coronary flow rate in isolated hearts from all pretreatment groups. Decreases in heart rate and rate pressure product, indicative of a cardiodepressant effect of cocaine, were seen in parallel to decreases in coronary flow rate. The latter may be a response to decreased metabolic demand due to the cardiodepressant activity or due to a direct vasoconstrictor action of cocaine on the coronary circulation. Our present data do not distinguish between these two possibilities. However, a direct vasoconstrictor action of cocaine is suggested by work by Vitullo et al. (1989), and by Chokshi et al. (1990) who demonstrated cocaine-induced vasoconstriction in isolated coronary vessels independent of adrenoceptors. In the present study, none of these effects were altered by nandrolone pretreatment.

In vivo, cocaine caused significant dose-related increases in heart rate. Consistent with the data of Pan and Hedaya (1998), cardiovascular effects of cocaine by the i.p. route in conscious rats were manifest at much higher concentrations than when cocaine is administered by the i.v. route. The increases in heart rate observed in the present study were potentiated by nandrolone only at the highest dose of cocaine used. The tachycardia induced by cocaine in vivo appears to be due to its sympathomimetic effect (Gillis et al., 1995). However, there is conflicting evidence as to whether cocaine mediates an increase in heart rate via a peripheral effect on neuronal uptake sites for noradrenaline (Iversen, 1963) in the heart, or via a central effect which in turn enhances sympathetic outflow to the heart (Wilkerson, 1988; Kiritsy-Roy et al., 1990).

The mechanism of the potentiation of cocaine's action on heart rate by nandrolone in the present study was not established. However, it may involve the inhibition of extraneuronal uptake of noradrenaline, which has been shown to be inhibited by other androgenic–anabolic steroids in isolated rat hearts (Salt, 1972). Thus, a potential combined effect of cocaine on neuronal and nandrolone on extraneuronal uptake sites for noradrenaline, could have resulted in a large increase in extracellular noradrenaline. However, nandrolone pretreatment had no significant effect on heart rate in vivo in the absence of cocaine or in the presence of lower doses of cocaine. It is possible that blockade of extraneuronal uptake of noradrenaline by nandrolone may have become more significant at higher extracellular concentrations of noradrenaline associated with high dose cocaine treatment. Anabolic steroids are also known to have effects on the central nervous system (CNS) as evidenced by their ability to induce both clinical and experimental aggression (Pope and Katz, 1994; Kashkin and Kleber, 1989). It is not known whether anabolic steroid can modulate cardiac function through the CNS. The central effect of cocaine results from neuronal blockade of serotonin, dopamine and noradrenaline reuptake and the release of catecholamines from the adrenal medulla (Kennedy and Hanbauer, 1983).

The data of Lomax and Daniel (1990) are consistent with the inability of 15 mg/kg of cocaine to induce a significant increase in body temperature in the present study. Lomax and Daniel demonstrated that 20 mg/kg (i.p.) cocaine was not sufficient to cause an increase in core temperature in exercising rats. It was also later reported that high ambient temperature and exercise dramatically increase the ability of cocaine to induce hyperthermia (Lomax and Daniel, 1993). Intravenous injection of cocaine has been reported to have no effect on oral temperature in humans under normal ambient temperature at rest (Clark and Lipton, 1986). Radiotelemetry allows an accurate reflection of changes in temperature. Dafters (1994) demonstrated that rectal temperature probes can cause stress-induced hyperthermia up to 1°C.

This is the first study to utilise radiotelemetry to measure ECG changes in response to cocaine in the rat. It was found that 45 mg/kg of cocaine, in comparison to saline, caused a significant ($P < 0.05$) prolongation of the QTc interval in both nandrolone and, to a lesser extent, vehicle pretreated rats, without a significant effect upon PR or QRS. These changes in the QTc interval were observed in the absence of arrhythmia in all pretreatment groups. Prolongation of the QTc interval in this study probably reflects regional cardiac differences in the rate of repolarisation rather than an effect on QRS. Grossie (1993) suggested that an effect on repolarisation can be attributed to the action of cocaine against calcium-dependent potassium channels. Nandrolone pretreatment caused a potentiation of the QTc prolongation by cocaine, but this potentiation did not occur consistently at all time points. The present study found no evidence of cardiac arrhythmias following cocaine administration by the i.p. route. A number of studies have observed cardiac arrhythmia following administration of cocaine by the i.v. route in the rat (Schwartz et al., 1989) and in dogs (Temesy-Armos et al., 1992). However, investigation of the cardiac effects of cocaine by the i.p. route in the rat remains clinically relevant since Javaid and Davis (1993) demonstrated that the disposition pattern of cocaine in rats after i.p. administration was similar to that observed in humans after intranasal administration.

In conclusion, this study has shown that short-term, high dose nandrolone administration in fully grown, normotensive, male, albino Wistar rats is not sufficient to significantly alter heart rate. However, this investigation has shown for the first time that this same dose of nandrolone potentiates the heart rate response to 45 mg/kg cocaine. Further investigation is required to determine the exact mechanism of this potentiation. High dose, short-term nandrolone administration was found to be insufficient to cause cardiac hypertrophy in adult rats. This study has also found that short-term exposure to high dose nandrolone and subsequent i.p. administration of cocaine does not induce cardiac arrhythmia or cause significant ECG changes.

Acknowledgements

The authors wish to express sincere appreciation to D. Taverner for assistance in computer programming and to Organon Pharmaceuticals for the kind donation of nandrolone decanoate. This research was supported in part by the Australian Research Council.

References

Besse, S., Assayag, P., Latour, C., Janmot, C., Robert, V., Delcayre, C., Nahas, G., Swynghedauw, B., 1997. Molecular characteristics of cocaine-induced cardiomyopathy in rats. Eur. J. Pharmacol. 338, 123–129.

Billman, G.E., 1990. Mechanisms responsible for the cardiotoxic effects of cocaine. FASEB J. 4, 2469–2475.

Billman, G.E., 1995. Cocaine: a review of its toxic actions on cardiac function. Crit. Rev. Toxicol. 25, 113–132.

Billman, G.E., Lappi, M.D., 1993. Effects of cocaine on cardiac vagal tone before and during coronary artery occlusion: cocaine exacerbates the autonomic response to myocardial ischemia. J. Cardiovasc. Pharmacol. 22, 869–876.

Brickner, M.E., Willard, J.E., Eichhorn, E.J., Black, J., Grayburn, P.A., 1991. Left ventricular hypertrophy associated with chronic cocaine abuse. Circulation 84, 1130–1135.

Brockway, B.P., Mills, P.A., Azar, S.H., 1991. A new method for continuous chronic measurement and recording of blood pressure, heart rate and activity in the rat via radiotelemetry. Clin. Exp. Hypertens., Part A: Theory Pract. 13, 885–895.

Chokshi, S.K., Gal, D., DeJesus, S., Isner, J.M., 1990. Cocaine produces vasoconstriction of human coronary arteries: in vitro studies using

coronary arteries obtained from freshly explanted human hearts. J. Am. Coll. Cardiol. 15, 215A.

Clark, W.G., Lipton, J.M., 1986. Changes in body temperature after administration of adrenergic and serotonergic agents and related drugs including antidepressants: II. Neurosci. Biobehav. Rev. 10, 153–220.

Cornish, J.W., O'Brien, C.P., 1996. Crack cocaine abuse: an epidemic with many public health consequences. Annu. Rev. Public Health 17, 259–273.

Dafters, R.I., 1994. Effect of ambient temperature on hyperthermia and hyperkinesis induced by 3,4 methylendioxymethamphetamine (MDMA or ''ecstasy'') in rats. Psychopharmacology 114, 505–508.

DuRant, R.H., Rickert, V.I., Ashworth, C.S., Newman, C., Slavens, G., 1993. Use of multiple drugs among adolescents who use anabolic steroids. N. Engl. J. Med. 328, 922–926.

DuRant, R.H., Escobedo, L.G., Heath, G.W., 1995. Anabolic steroid use, strength training and multiple drug use among adolescents in the United States. Pediatrics 96, 23–28.

Gillis, R.A., Hernandez, Y.M., Erzouki, H.K., Raczkowski, V.F., Mandal, A.K., Kuhn, F.E., Dretchen, K.L., 1995. Sympathetic nervous system mediated cardiovascular effects of cocaine are primarily due to a peripheral site of action of the drug. Drug Alcohol Depend. 37, 217–230.

Grossie, J., 1993. Ca2+ dependent action of cocaine on K+ current in freshly dissociated dorsal root ganglia from rats. Am. J. Physiol. 265, C674–C679.

Irvine, R.J., White, J.M., Chan, R., 1997. The influence of restraint on blood pressure in the rat. J. Pharmacol. Toxicol. Methods 38, 157–162.

Iversen, L.L., 1963. The uptake of noradrenaline by the isolated perfused rat heart. Br. J. Pharmacol. 21, 523–537.

Javaid, J.I., Davis, J.M., 1993. Cocaine disposition in discrete regions of rat brain. Biopharm. Drug Dispos. 14, 357–364.

Kabas, J.S., Blanchard, S.M., Matsuyama, Y., 1990. Cocaine mediated impairment of cardiac conduction in the dog: a potential mechanism for sudden death after cocaine. J. Pharmacol. Exp. Ther. 252, 185–191.

Karch, S.B., Green, S.G., Young, S.N., 1995. Myocardial hypertrophy and coronary artery disease in male cocaine users. J. Forensic Sci. 40, 591–595.

Kashkin, K.B., Kleber, H.D., 1989. Hooked on hormones? An anabolic steroid addiction hypothesis. JAMA 262, 3166–3170.

Kennedy, L.T., Hanbauer, I., 1983. Sodium sensitive cocaine binding to rat striatal membrane: possible relationship to dopamine uptake sites. J. Neurochem. 41, 172–178.

Kennedy, M.C., Lawrence, C., 1993. Anabolic steroid abuse and cardiac death. Med. J. Aust. 158, 346–348.

Kinson, G.A., Layberry, R.A., Hebert, B., 1991. Influences of anabolic androgens on cardiac growth and metabolism in the rat. Can. J. Physiol. Pharmacol. 69, 1698–1704.

Kiritsy-Roy, J.A., Halter, J.B., Gordon, S.M., Smith, M.J., Terry, L.C., 1990. Role of the CNS in haemodynamics and sympathoadrenal response to cocaine in rats. J. Pharmacol. Exp. Ther. 255, 154–160.

Koenig, H., Goldstone, A., Lu, C., 1982. Testosterone-mediated sexual dimorphism of the rodent heart. Circ. Res. 50, 782–787.

Lange, R.A., Cigarroa, R.G., Yancy, C.W., Wilard, J.E., Popma, J.J., Sills, M.N., McBride, W., Kim, A.S., Hillis, L.D., 1989. Cocaine induced coronary artery vasoconstriction. N. Engl. J. Med. 321, 1557–1562.

Lomax, P., Daniel, K.A., 1990. Cocaine and body temperature in the rat: effects of ambient temperature. Pharmacology 40, 103–109.

Lomax, P., Daniel, K.A., 1993. Cocaine and body temperature: effects of exercise at high ambient temperature. Pharmacology 46, 164–172.

Luke, J.L., Farb, A., Virmani, R., Sample, R.H., 1990. Sudden cardiac death during exercise in a weight lifter using anabolic–androgenic steroids: pathological and toxicological findings. J. Forensic Sci. 35, 1441–1447.

Marsh, J.D., Lehmann, M.H., Ritchie, R.H., Gwathmey, J.K., Green, G.E., Schiebinger, R.J., 1998. Androgen receptors mediate hypertrophy in cardiac myocytes. Circulation 98, 256–261.

McKillop, G., Todd, I.C., Ballantyne, D., 1986. Increased left ventricular mass in a bodybuilder using anabolic steroids. Br. J. Sports Med. 20, 151–152.

Pan, W.-J., Hedaya, M.A., 1998. An animal model for simultaneous pharmacokinetic/pharmacodynamic investigations: application to cocaine. J. Pharmacol. Toxicol. Methods 39, 1–8.

Perreault, C.L., Hague, N.L., Ransil, B.J., Morgan, J.P., 1990. The effects of cocaine on intracellular Ca^{2+} handling and myofilament Ca^{2+} responsiveness of ferret myocardium. Br. J. Pharmacol. 101, 679–685.

Pitts, D.K., Marwah, J., 1989. Autonomic actions of cocaine. Can. J. Physiol. Pharmacol. 67, 1168–1176.

Pope, H.G., Katz, D.L., 1994. Psychiatric and medical effects of anabolic–androgenic steroid use. Arch. Gen. Psychiatry 51, 375–382.

Reiter, M., 1988. Calcium mobilisation and cardiac inotropic mechanisms. Pharmacol. Rev. 40, 189–218.

Salt, P.J., 1972. Inhibition of noradrenaline uptake-2 in the isolated rat heart by steroids, clonidine and methoxylated phenylethylamines. Eur. J. Pharmacol. 20, 329–340.

Schwartz, A.B., Janzen, D., Jones, R.T., Boyle, W., 1989. Electrocardiographic and hemodynamic effects of intravenous cocaine in awake and anesthetized dog. J. Electrocardiol. 22 (2), 159–166.

Shakalis, A., Condouris, G.A., 1967. Local anesthetic action of cocaine on nerves from adrenalectomized frogs. Arch. Int. Pharmacodyn. Ther. 166, 93–101.

Simkhovitch, B.Z., Kloner, R.A., Alker, K.J., Giaconi, J., 1994. Time course of direct cardiotoxic effects of high cocaine concentration in isolated rabbit heart. J. Cardiovasc. Pharmacol. 23, 509–516.

Stewart, G., Rubin, E., Thomas, A.P., 1991. Inhibition by cocaine of excitation–contraction coupling in isolated cardiomyocytes. Am. J. Physiol. 260, H50–H57.

Temesy-Armos, P.N., Fraker, T.D., Brewster, P.S., Wilkerson, R.D., 1992. The effects of cocaine on cardiac electrophysiology in conscious, unsedated dogs. J. Cardiovasc. Pharmacol. 19, 883–891.

Tseng, Y.T., Rockhold, R.W., Hoskins, B., Ho, I.K., 1994. Cardiovascular toxicities of nandrolone and cocaine in spontaneously hypertensive rats. Fundam. Appl. Toxicol. 22, 113–121.

Vitullo, J.C., Karam, R., Mekhail, N., Wicker, P., Engelmann, G.l., Khairallah, P.A., 1989. Cocaine-induced small vessel spasm in isolated rat hearts. Am. J. Pathol. 135, 85–91.

Wilkerson, R.D., 1988. Cardiovascular effects of cocaine in conscious dogs: importance of fully functional autonomic and central nervous systems. J. Pharmacol. Exp. Ther. 246, 466–471.

Yesalis, C.E., Kennedy, N.J., Kopstein, A.N., Bahrke, M.S., 1993. Anabolic–androgenic steroid use in the Unite States. JAMA 270, 1217–1221.