Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 464 (2003) 189–196
www.elsevier.com/locate/ejphar

Hemodynamic response profile predicts susceptibility to cocaine-induced toxicity

John B. Williams, Susan M. Keenan, Qi Gan, Mark M. Knuepfer*

Department of Pharmacological and Physiological Science, Saint Louis University School of Medicine, 1402 S. Grand Boulevard, St. Louis, MO 63104, USA

Received 4 December 2002; accepted 4 February 2003

Abstract

Cocaine evokes pressor responses due either to a large increase in systemic vascular resistance despite a decrease (>8%) in cardiac output (vascular responders) or to small increases in both cardiac output and vascular resistance (mixed responders) in conscious rats. These studies were designed to determine (1) if the hemodynamic response pattern to cocaine correlates with relative sensitivity to toxicity and (2) if altering the hemodynamic response pattern to cocaine using propranolol enhances toxicity. Rats were instrumented for determination of cardiac output and arterial pressure. After recovery, rats were classified as vascular or mixed responders to cocaine (5 mg/kg, i.v., four to six trials). Two weeks later, cocaine was infused (1.5 mg/kg/min) until death after pretreatment with saline or propranolol (1 mg/kg). Saline-pretreated mixed responders ($n=6$) had greater tolerance to cocaine toxicity compared to vascular responders ($n=11$). Furthermore, saline-pretreated vascular responders were less sensitive than propranolol-pretreated vascular responders ($n=9$) to cocaine toxicity. Therefore, we propose that the initial hemodynamic response pattern to cocaine predicts sensitivity to cocaine toxicity. In addition, propranolol, a drug that enhances the increase in vascular resistance to cocaine, also increases toxicity to cocaine in vascular responders.

Keywords: Cocaine; Cardiac output; Arterial pressure

1. Introduction

Cocaine use in human beings leads to cardiac abnormalities such as myocardial ischemia, infarction, arrhythmias, sudden cardiac death and cardiomyopathies. Several researchers have noted a lack of a clear dose–response relationship in the occurrence of cocaine-induced apparent myocardial ischemia (Amin et al., 1990; Minor et al., 1991), coronary vasoconstriction (Flores et al., 1990; Lange et al., 1989, 1990), cardiomyopathies (Karch et al., 1998; Minor et al., 1991) and morbidity (Mittleman and Wetli, 1987; Smart and Anglin, 1987). These findings suggest that, in humans, certain individuals are at a greater risk for severe cocaine-induced cardiovascular complications than others. Therefore, studies examining toxicity that identify individuals at high or low risk for cocaine-induced cardiovascular complications would be clinically relevant.

We noted differences in the hemodynamic response patterns to cocaine in a number of studies (Branch and Knuepfer, 1992, 1994a; Knuepfer et al., 1993, 2001). Cocaine administration evokes a pressor response either by evoking a substantial increase in systemic vascular resistance (with a decrease in cardiac output) or by evoking a smaller increase in systemic vascular resistance and a small increase or no change in cardiac output. We arbitrarily divided rats into two subsets based on their cardiac output response to cocaine to identify the significance of the varying response profiles. Due to these cardiovascular response profiles, we refer to these subsets as vascular and mixed responders, respectively. These responses are consistent and reproducible within individuals. Vascular responders have greater susceptibility to cocaine-induced cardiomyopathies and a sustained elevation in arterial pressure (Branch and Knuepfer, 1994a; Knuepfer et al., 1993). Therefore, we proposed that vascular responders represent a subset of the population predisposed to cardiovascular disease possibly similar to humans that appear to be more sensitive to cocaine-induced toxicity (Knuepfer and Mueller, 1999).

* Corresponding author. Tel.: +1-314-577-8542; fax: +1-314-577-8233.
E-mail address: knuepfmm@slu.edu (M.M. Knuepfer).

0014-2999/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0014-2999(03)01429-8

While several investigators have noted that cocaine produces hemodynamic responses due to sympathetic activation (Branch and Knuepfer, 1994a; Knuepfer and Branch, 1992; Poon and van den Buuse, 1998), our data suggest that vascular responders may have more robust sympathetic responses to cocaine (Branch and Knuepfer, 1994b; Purcell et al., 2001). We suggested that this may be responsible for the differences in cardiac output and systemic vascular resistance responsiveness (Knuepfer and Mueller, 1999). These hemodynamic characteristics are not unique to cocaine since conditioned or unconditioned behavioral stress, amphetamine administration or ethanol administration evoke similar differential cardiovascular responses (Branch and Knuepfer, 1994a; Knuepfer et al., 2001; Mueller et al., 1997; Muller et al., 2001). Therefore, the variability in responsiveness to cocaine may be a generalized response to acute behavioral arousal that is mediated by greater sympathetic responsiveness (Knuepfer and Mueller, 1999).

The β-adrenoceptor antagonist propranolol has been used clinically to treat individuals with symptoms of cocaine-induced cardiovascular toxicity (Gay, 1982; Rappolt et al., 1977). There is increasing clinical and experimental evidence that propranolol may not be beneficial in patients who are suffering cardiovascular complications after cocaine use and may, in fact, be detrimental by enhancing coronary vasoconstriction (Lange et al., 1990; Ramoska and Sacchetti, 1985). Propranolol pretreatment enhances the decrease in cardiac output in vascular responders and causes a decrease in mixed responders and exacerbates the increase in systemic vascular resistance, enhancing the cocaine-induced coronary vasoconstriction, resulting in a greater pressor response to cocaine (Branch and Knuepfer, 1994a; Knuepfer et al., 1998). Therefore, we predicted that propranolol pretreatment followed by a lethal cocaine infusion should enhance toxicity.

We determined if differential cardiac output responses to cocaine correlates with relative sensitivity to the onset of immobility, seizures and death using a common model of cocaine toxicity (Catravas and Waters, 1981; Derlet and Albertson, 1990; Guinn et al., 1980; Heavner et al., 1995; Mets et al., 1996; Tella et al., 1992; Trouvé and Nahas, 1990; Wilson and Holbrook, 1978; Witkin et al., 1989). In addition, we examined the role of β-adrenoceptors in modifying cocaine-induced toxicity. Our data suggests that the hemodynamic response patterns correlate with individual predisposition to cocaine toxicity in rats and that the toxicity is enhanced by propranolol.

2. Methods

This study required two periods in which individual animals were studied. Initially, animals were instrumented for characterization of hemodynamic response profile to cocaine. Subsequently, rats were allowed to recover for 2–10 weeks to ensure that lasting effects of cocaine did not affect the toxicity studies. After complete recovery, we recannulated rats (since arterial cannulas did not remain patent) for subsequent studies on the hemodynamic effects of lethal cocaine infusions. This was done in a different location to avoid reopening any healed wounds.

All surgical and experimental procedures were approved by the Saint Louis University Institutional Animal Care and Use Committee and followed guidelines described in the ''Guide for the Care and Use of Laboratory Animals'' (National Research Council, National Academy Press, Washington, DC, 1996).

2.1. Animal preparation

Using aseptic technique, male Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN), weighing 300–425 g, were surgically prepared under sodium pentobarbital (50 mg/kg) anesthesia (Branch and Knuepfer, 1992, 1994; Knuepfer and Branch, 1992; Knuepfer et al., 1993, 1998). A thoracotomy was performed to place a miniaturized pulsed Doppler flow probe (Iowa Doppler Products, Iowa City, IA) filled with acoustic gel on the ascending aorta. After closing the thorax with sutures, lead wires were brought subcutaneously to a socket in the skull. When the surgery was completed, rats were treated with cefazolin (10 mg/kg, i.m.) and allowed to recover for 7–10 days.

Following recovery, rats were anesthetized with a mixture of ketamine and xylazine (55 and 7 mg/kg, respectively). Arterial and venous cannulas were implanted in the left femoral artery and vein, respectively, using Tygon Microbore® tubing. The tubing was led subcutaneously to the nape of the neck and externalized. Cannulas were filled with 200 U/ml of heparin. In addition, wire leads (0.003 in. diameter S/S) were implanted subcutaneously into the right and left forelimb and the left hindlimb. These were tunneled subcutaneously to the head and fixed to the skull adjacent to the flow probe socket with dental cement.

One or two days later, rats were acclimated in a Plexiglas cage ($31 \times 31 \times 35$ cm high) for at least 6 h. On the following day, rats were placed in the same cage for at least 2 h before beginning experimentation.

2.2. Response characterization

The procedures used to characterize rats as vascular or mixed responders have been described in previous reports (Branch and Knuepfer, 1992, 1994a4; Knuepfer and Branch, 1992; Knuepfer et al., 1993, 1998). Briefly after acclimation to the test cage, rats were treated with cocaine (0.5 mg/kg, i.v., four to six trials) on the first day of testing. Doses were administered 1–2 h apart to avoid tachyphylaxis (Branch and Knuepfer, 1994a; Knuepfer and Branch, 1992). On the following day, rats were acclimated again for at least 1 h in the test cage. Subsequently, rats were treated with cocaine (5 mg/kg, i.v., twice daily) with an interdosing

interval of at least 3 h. This procedure was repeated for the next day or two such that each rat had four to six trials with the higher dose of cocaine. We did not observe tachyphylaxis of cardiovascular responses to cocaine using these dosing intervals as previous reported (Branch and Knuepfer, 1994a).

Arterial pressure, heart rate and cardiac output were monitored before and for 5 min after cocaine administration. Percent changes in cardiac output were determined from the shift in Doppler frequency. Rats were classified as vascular or mixed responders according to the maximum change in cardiac output after each trial. If the mean change in cardiac output from all trials was greater than −8%, rats were classified as mixed responders. Those with greater decreases were designated vascular responders. These criteria were developed due to the distribution of mean cardiac output responses to cocaine noted in previous studies (Branch and Knuepfer, 1992, 1994a; Knuepfer and Branch, 1992; Knuepfer et al., 1993, 1998).

Percent changes in systemic vascular resistance were calculated by dividing the arterial pressure by the cardiac output. Changes in stroke volume were determined by dividing the cardiac output by the heart rate. The maximum change in the cardiac output after injection, either positive or negative, was used to classify rats as vascular or mixed responders, respectively. All experiments were performed between the hours of 8:30 a.m. and 4:30 p.m. in a quiet room. A minimum of 2 weeks was allowed for recovery after characterization of hemodynamic responses.

2.3. Cocaine infusion protocol

Following the recovery period, the contralateral femoral artery and vein were recannulated under ketamine-xylazine (55 and 7 mg/kg, respectively) anesthesia and using cefaxolin (10 mg/kg, i.m.) to prevent sepsis. Cannulas were externalized as before but not through the same incision made for the first set of cannulae. Recannulation was necessary due to the loss of patency of chronic implanted vascular cannulas. In contrast, we were able to maintain the cardiac output signal in these rats. One to two days later, rats were reacclimated to test cages for at least 6 h. The next day, rats were acclimated in their test cages for at least 1 h.

One group of rats ($n=22$) were infused with cocaine at 1.5 mg/kg/min following a saline bolus pretreatment (1 ml/kg, i.v.). Arterial pressure, heart rate, ECG and cardiac output were recorded. Cocaine infusion was continued until cardiac arrest. Hemodynamic parameters were displayed on a chart recorder and digitized and stored on a personal computer. Several events were recorded including the time of the first seizure (>2 s in duration), the time of initial enervation (prostration on the cage bottom) and the time of death (defined as an arterial pressure <30 mm Hg) were recorded. After death, the heart was removed rapidly, fixed in 10% formalin and later sectioned for histological analysis.

Another group of rats classified as vascular responders ($n=9$) received propranolol (1 mg/kg) as a pretreatment, 10 min prior to the cocaine infusion. These rats were randomly selected from those rats previously classified with cocaine. Hemodynamic parameters were measured before and after drug administration and during cocaine infusion until death.

2.4. Materials

Materials used included propranolol hydrochloride from Sigma (St. Louis, MO). Cocaine hydrochloride was obtained from the National Institute on Drug Abuse. Drugs were dissolved in 0.9% sterile saline and were administered intravenously in a final volume of 1 ml/kg. Drug concentrations were calculated as the salt form. Ketamine (Ketaset III®) was obtained from Fort Dodge Pharmaceuticals (Fort Dodge, IA) and xylazine (Rompun®) was obtained from Bayer, Agricultural Division (Shawnee Mission, KS). Cefazolin (Geneva Pharmaceuticals/Marsam Pharmaceuticals, Cherry Hill, NJ) was used to reduce the risk of sepsis following surgery.

Table 1
Baseline hemodynamic values and responses to cocaine alone

	N	MAP resting (mm Hg)	MAP change (mm Hg)	HR resting (b/m)	HR change (b/m)	CO resting (kHz shift)	CO change (%)	SVR change (%)	SV change (%)
Saline and cocaine (0.5 mg/kg)									
Mixed responders	9	132.5 (2.9)	22.6 (2.9)	423 (11)	−16 (14)	9.7 (0.6)	11.2 (2.2)	6.0 (3.4)	17.8 (4.3)
Vascular responders	11	126.6 (4.4)	25.7 (5.6)	445 (33)	−6 (29)	8.9 (0.6)	−4.2**	28.8** (6.8)	3.3* (8.8)
Saline and cocaine (5 mg/kg)									
Mixed responders	10	131.1 (6.8)	34.2 (4.0)	427 (9)	−40 (11)	9.7 (0.7)	3.5 (3.3)	28.4 (6.5)	11.0 (3.7)
Vascular responders	12	123.0 (3.1)	33.9 (4.8)	407 (23)	−40 (35)	9.2 (0.6)	−20.3** (3.1)	61.3** (9.6)	−7.1** (4.6)
Propranolol and cocaine (5 mg/kg)									
Vascular responders	9	118.5 (2.6)	33.7 (5.2)	407 (22)	−77 (35)	10.3 (0.8)	−17.4** (2.2)	65.2** (7.0)	−8.8* (3.0)

Parameters are mean arterial pressure (MAP), heart rate (HR), cardiac output (CO), systemic vascular resistance (SVR) and stroke volume (SV).
Data shown are mean ± S.E.M. (in parentheses).
*$p<0.05$ compared to mixed responders using a Student's t-test.
**$p<0.005$ compared to mixed responders using a Student's t-test.

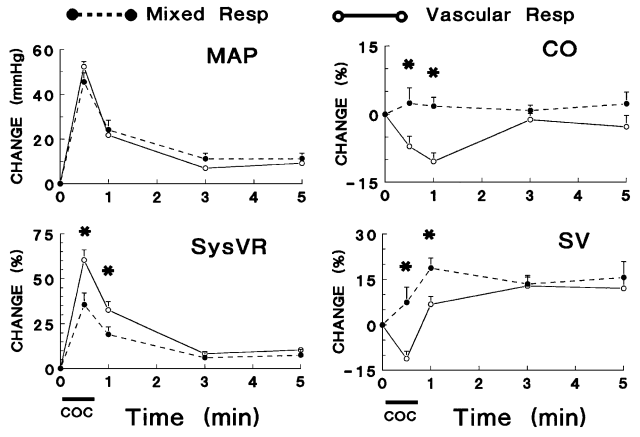

Fig. 1. Responses to acute cocaine administration (5 mg/kg, i.v., over 45 s) in vascular and mixed responders. Abbreviations include: MAP, mean arterial pressure; CO, cardiac output; SV, stroke volume; SysVR, systemic vascular resistance; Coc, cocaine. Responses were compared between groups using a two-way ANOVA and Newman–Keuls post-hoc comparison to note differences between individual values. Significant differences ($p<0.05$) between groups are noted with an asterisk. The vascular responders include animals that were later pretreated with saline or propranolol for the infusion studies since responses were not different (Table 1).

2.5. Data analysis

During the classification stage of the experiment, data was analyzed at several time points. First, the peak in arterial pressure after administration of cocaine was used to identify the initial responses. This response occurred within the first 45 s, invariably. Second, a set of values were taken at the maximum change in cardiac output if it was not coincident with the peak change in arterial pressure. Additionally, points were taken during the sustained, modest pressor response at 1, 3 and 5 min after injecting cocaine. Peak data were analyzed by two-way analysis of variance (ANOVA), whereas the time course (1, 3 and 5 min) was analyzed by three-way ANOVA.

Due to the wide variability in toxic doses of cocaine between rats, cocaine infusion data were analyzed at several time points representing a percent time until death. Changes in hemodynamic values were compared with analysis of variance using a Newman–Keuls post-hoc test to analyze differences at specific times. Vascular responders with and without propranolol pretreatment were compared similarly.

The differences in time of initial seizure, enervation and death were analyzed with a Wilcoxon rank sum/Mann–Whitney *U*-test to determine differences between individual groups.

3. Results

3.1. Hemodynamic responses to cocaine

After acclimation to the test cage, rats treated with cocaine alone had a mean arterial pressure of 122 ± 2 mm

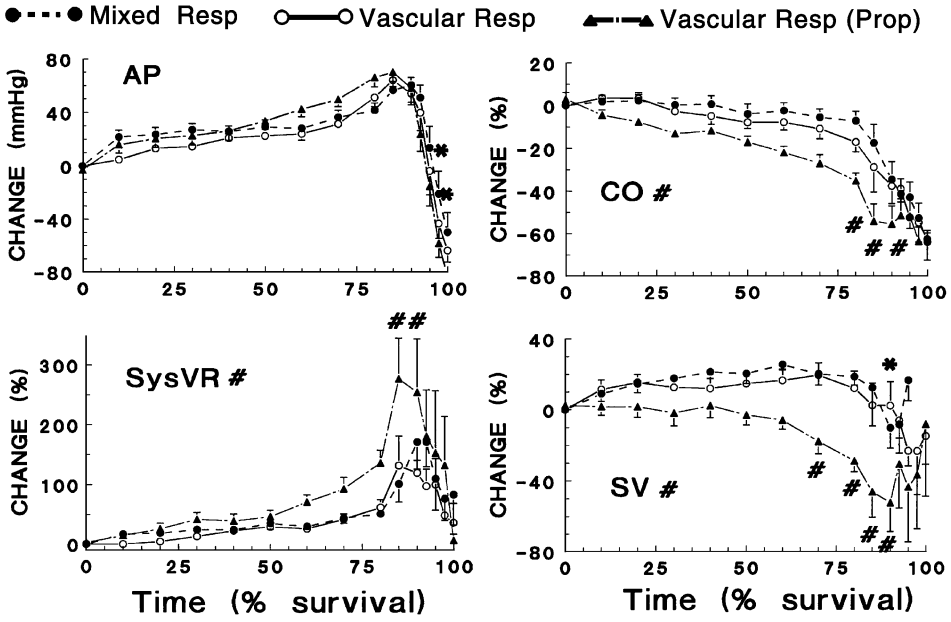

Fig. 2. The effects of continuous cocaine infusion (1.5 mg/kg/min, i.v.) on hemodynamic responses are shown. In order to compare groups with different survival times, data were compared by determining responses at different percent times until death. In this manner, differences in survival times did not alter the ability to compare rats during the lethal infusion. Abbreviations are described in Fig. 1. Data were compared with a two-way ANOVA using Newman–Keuls post-hoc test to determine differences at each time point on the curves. Significant differences between saline pretreated vascular and mixed responders are noted with an asterisk. Significant differences between saline pretreated vascular responders and propranolol pretreated animals are noted with a #. Furthermore, overall differences using all time points, determined by ANOVA, were noted in cardiac output, systemic vascular resistance and stroke volume. These are signified by a # adjacent to the abbreviation.

Hg, a heart rate of 417 ± 7 b/min and an ascending aortic flow of 9.7 ± 0.3 kHz shift. There were no significant differences in the resting values of vascular and mixed responders (Table 1). Likewise, there were no differences in resting values between vascular responders with saline pretreatment and those pretreated with propranolol (Table 1). Administration of cocaine (0.5 mg/kg, i.v.) elicited an increase in arterial pressure associated with variable changes in cardiac output, systemic vascular resistance and stroke volume. There were significant differences between vascular and mixed responders in the cardiac output, systemic vascular resistance and the stroke volume responses at the time of the peak change in cardiac output (Table 1). The hemodynamic responses to acute cocaine (5 mg/kg, i.v.) treatment are shown in Fig. 1. The cardiac output, stroke volume and systemic vascular resistance responses to cocaine were significantly different between groups yet the mean arterial pressure and heart rate responses were not different (heart rate data not shown).

3.2. Effects of lethal cocaine infusions

Cocaine infusion (1.5 mg/kg/min) elicited increases in arterial pressure and resulted in death in less than 46 min in all 17 rats. The hemodynamic responses from mixed ($n = 9$) and vascular responders ($n = 11$) are plotted on a percent time until death as depicted in Fig. 2. There were no differences in hemodynamic responsiveness to a slow infusion of cocaine. Arterial pressure, stroke volume and systemic vascular resistance rose initially while cardiac output and heart rate (data not shown) fell slowly. When near death, all parameters fell precipitously presumably due to cardiac failure.

The relative time until the first seizure, immobility and death are compared in Fig. 3. Vascular responders ($n = 12$) died sooner than mixed responders ($n = 10$) but did not have seizures or become immobile sooner than mixed responders. Vascular responders died at significantly lower doses than mixed responders (35.8 ± 3.8 vs. 50.8 ± 5.1 mg/kg, respectively, $p < 0.02$). Fig. 4 shows the survival curve for vascular and mixed responders to compare the rates of death.

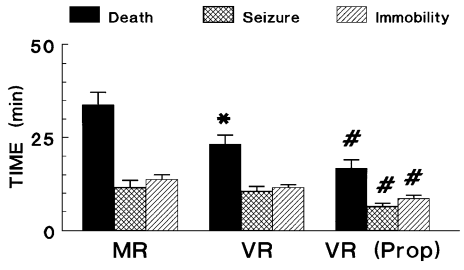

Fig. 3. The time until death, first seizure and to immobility in response to lethal cocaine infusion in vascular and mixed responders pretreated with saline (VR and MR, respectively) and in vascular responders pretreated with propranolol (1 mg/kg, i.v.). Significant differences between groups are denoted as in Fig. 2.

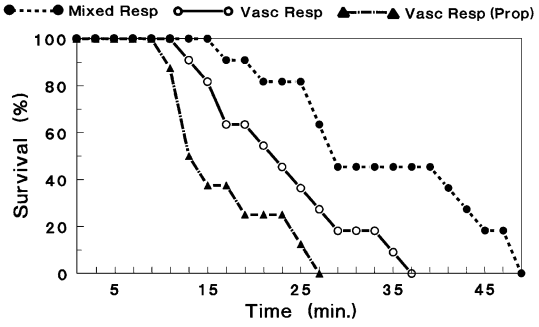

Fig. 4. Survival curves for each group of rats are compared. Differences between groups are noted in Fig. 3 and in the text.

3.3. Effects of propranolol pretreatment

Vascular responders to cocaine that were pretreated with propranolol ($n = 9$) had similar hemodynamic responses to cocaine alone (before cocaine infusion) as vascular responders pretreated with saline (Table 1). Because the saline-pretreated vascular responders were not different from the vascular responders later pretreated with propranolol, these data are combined in Fig. 1. In response to cocaine infusion, hemodynamic responses of vascular responders receiving saline pretreatment and those receiving propranolol pretreatment are plotted in Fig. 2. Propranolol pretreatment did not alter the arterial pressure responses to cocaine infusion but significantly elevated the increase in systemic vascular resistance and depressed the cardiac output and systemic vascular resistance changes (Fig. 2).

Propranolol pretreatment also reduced the time until death, the first seizure and immobilization compared to saline-treated vascular responders (Figs. 3 and 4). Propranolol-pretreated vascular responders died at significantly lower doses than untreated vascular responders (25.2 ± 3.4 and 35.8 ± 3.8 mg/kg, respectively, $p = 0.026$).

Microscopic examination of the myocardia after lethal infusion did not reveal any abnormalities suggestive of ischemia, infarction, congenital abnormalities, infectious causes of cardiac dysfunction or other signs of preexisting alterations at the light microscopic level.

4. Discussion

Our results demonstrate a direct relationship between the acute hemodynamic response pattern to cocaine and the susceptibility to cocaine toxicity. Mixed responders were more resistant to cocaine-induced toxicity compared to vascular responders. Many investigators have used similar models of cocaine toxicity (Catravas and Waters, 1981; Derlet and Albertson, 1990; Guinn et al., 1980; Heavner et al., 1995; Mets et al., 1996; Tella et al., 1992; Trouvé and Nahas, 1990; Wilson and Holbrook, 1978; Witkin et al., 1989). Typically, others have noted substantial variation in

the dose of cocaine necessary to produce lethality in single strains, although most have not been able to predict which animals are at risk. Recently, Shi et al. (1999) reported that a strain of rats more prone to kindling-induced seizures are more resistant to cocaine-induced toxicity compared to rats bred to be resistant to kindling-induced seizures. These data suggest that certain phenotypes can be identified that covary with the individual susceptibility to cocaine-induced toxicity. To our knowledge, this is the first demonstration that a single strain can be subdivided by any parameter into those more or less susceptible to cocaine toxicity. In other words, we have identified a novel risk factor for predisposition to cocaine toxicity. We previously reported that vascular responders had more severe cardiomyopathies in response to repeated cocaine compared to mixed responders (Knuepfer et al., 1993) and had a predisposition to a sustained elevation in arterial pressure after repeated cocaine treatment (Branch and Knuepfer, 1994a). The present findings provide additional evidence that this subset of the population is predisposed to cocaine-induced toxicity (Branch and Knuepfer, 1994a; Knuepfer and Mueller, 1999).

Several observations suggest that susceptibility to cocaine-induced cardiotoxicity varies widely in humans. Clinical studies have described a poor dose–response relationship with respect to the occurrence of myocardial ischemia and related electrocardiographic alterations (Amin et al., 1990; Minor et al., 1991), coronary vasoconstriction (Flores et al., 1990; Lange et al., 1989, 1990), cardiomyopathies (Isner et al., 1986; Karch et al., 1998; Minor et al., 1991; Smart and Anglin, 1987) and to morbidity (Mittleman and Wetli, 1987; Smart and Anglin, 1987; Wetli and Wright, 1979). Furthermore, the symptoms preceding lethality vary greatly in individuals (George, 1991; Minor et al., 1991; Ruttenber et al., 1997). It is clear from these data that various toxic responses to cocaine are noted in a fraction of those individuals using cocaine but we are unable to predict which individuals are at risk. We have suggested that our model of variable hemodynamic responsivity and susceptibility to toxicity in rats is related to that described in humans (Knuepfer and Mueller, 1999).

We suggested that vascular responders have greater increases in systemic vascular resistance due to greater sympathoexcitatory responses (Knuepfer and Mueller, 1999). We noted greater increases in renal sympathetic nerve activity in chloralose anesthetized or conscious vascular responders compared to mixed responders (Branch and Knuepfer, 1994b; Purcell et al., 2001). While it is not known what causes greater sympathoexcitatory responses, it is likely to be dependent on central corticotropin-releasing factor (CRF) since intracerebroventricular administration of CRF antagonists, α-helical CRF_{9-41} or astressin was capable of preventing the decrease in cardiac output and blunting the increase in systemic vascular resistance in vascular responders (Dong et al., 2001). Likewise, intranasal cocaine administration to human subjects produces sustained increases in sympathetic nerve activity that is buffered somewhat by baroreflex activity (Jacobsen et al., 1997; Vongpatanasin et al., 1999). The increases in sympathetic nerve activity were characterized by considerable variability as noted by large standard errors. Therefore, central neural pathways appear necessary for manifesting hemodynamic responses to cocaine and, possibly, the variability in responses.

Our results suggest that propranolol pretreatment enhances toxicity to cocaine in vascular responders. Similar results have been reported by others (Guinn et al., 1980; Tella et al., 1992) although the cardiac output and vascular resistances were not measured in these experiments. We reported that intravenous or intracerebroventricular administration of propranolol enhanced the decrease in cardiac output and increase in systemic vascular resistance in vascular responders and made mixed responders respond like vascular responders (Branch and Knuepfer, 1994a; Dong et al., 2001; Knuepfer et al., 1998). We suggested that the enhanced magnitude of the decrease in cardiac output and increase in systemic vascular resistance would increase susceptibility to cocaine-induced toxicity since the extent of abnormalities in cardiac myocytes was related to the decrease in cardiac output (Knuepfer et al., 1993). As predicted, propranolol pretreatment not only enhanced the decrease in cardiac output but also enhanced toxicity in vascular responders. Although we did not test mixed responders with propranolol, we predict that toxicity would also be enhanced in this group since this pretreatment prevents the increase in cardiac output normally noted in these animals and others have reported enhanced toxicity (Guinn et al., 1980; Smith et al., 1991; Tella et al., 1992). The enhanced systemic vascular resistance may also be due, in part, to prevention of cocaine-induced skeletal muscle vasodilation noted in earlier studies (Branch and Knuepfer, 1992).

Propranolol had been used clinically to treat acute cocaine toxicity (Gay, 1982; Rappolt et al., 1977) although other studies suggest it may potentiate toxicity (Guinn et al., 1980; Ramoska and Sacchetti, 1985) possibly by enhancing the vasoconstrictor action of cocaine (Lange et al., 1990). Our data suggests that this phenomenon may be mediated through an enhancement of the centrally mediated sympathoexcitatory effects of cocaine (Dong et al., 2001; Purcell et al., 2001). This, in turn could lead to greater systemic vasoconstriction and possibly reflex or direct depression of cardiac function. It is, as yet, unclear whether the primary effect of propranolol is to increase systemic vascular resistance or decrease cardiac output mediating the enhanced lethality to cocaine. It is not known whether β-adrenoceptor agonists or other direct inotropes alter cocaine toxicity, but it can be interpolated that if the protective effects noted after α-adrenergic blockade (Derlet and Albertson, 1990; Tella et al., 1992; Trouvé and Nahas, 1990) are a result of attenuating the decrease in systemic vascular resistance, then the toxic effects may be dependent on the increase in systemic vascular resistance. Likewise, we have shown that prazosin

prevents most of the hemodynamic effects of cocaine and eliminates the differences in hemodynamic responses between vascular and mixed responders (Branch and Knuepfer, 1992, 1994a).

Regarding the mechanism of the arterial pressure responses, our data demonstrate a progressive increase in arterial pressure during cocaine infusion due primarily to a rise in systemic vascular resistance and to a small increase in stroke volume (Fig. 2). The rise in systemic vascular resistance may be attributed to cocaine enhancing synaptic norepinephrine levels by inhibiting the synaptic reuptake of catecholamines. Greater catecholamine concentrations would increase α-adrenoceptor activation in the peripheral vasculature. We reported that propranolol augments the vasoconstrictor effects possibly by preventing skeletal muscle vasodilatation (Branch and Knuepfer, 1992). The gradual decrease in heart rate (data not shown) during the infusion may be due to the central sympathoinhibitory effects of cocaine on the heart or to a baroreflex response to the elevated arterial pressure. Cardiac output is maintained despite the declining heart rate possibly due to a longer diastolic phase enhancing stroke volume. Eventually the cardiac output and stroke volume begin to decrease as the rat appears to experience cardiac failure. Immediately before death, there was a brief pressor response presumably due to a sympathoexcitatory response. This coincided with a brief increase in heart rate and stroke volume further suggesting sympathoexcitation.

The first seizure and the onset of enervation (as noted by immobility and loss of upright position) during cocaine infusion occurred in the same order consistently and at similar times or doses for mixed and vascular responders. The lack of association with percent time till death in vascular and mixed responders suggests that the mechanism of cocaine-induced seizure and immobility is independent of the mechanism of lethality. George made a similar observation after noting that the ED_{50} for cocaine-induced seizures varied widely in several strains of mice whereas the LD_{50} for cocaine was relatively constant across strains (George, 1991).

Interestingly, propranolol pretreatment reduced the time of onset of seizures and immobility. Although it is not clear how propranolol altered the onset of these toxic responses, these findings are consistent with the observations of Tella et al. (1992). They reported that propranolol pretreatment significantly shortened the time to the first seizure noted during cocaine infusion. Mets et al. (1996) demonstrated that concomitant cocaine and catecholamine infusion enhanced the convulsive effects of a cocaine infusion suggesting that catecholamines contribute to the predisposition to seizures. The mechanisms are, as yet, unclear.

In summary, differences in hemodynamic response patterns predict the predisposition to cocaine toxicity. This was corroborated by enhancing the increase in systemic vascular resistance and decrease in cardiac output using propranolol. As predicted, toxicity was enhanced by this treatment. There is increasing clinical and experimental evidence as summarized above, that propranolol does not help in treating acute and chronically induced toxicity and may in fact be detrimental. We suggest that the differences are due to the sympathoexcitatory effects of cocaine. It is not clear whether sympathetic responses contribute to toxicity in humans but there is evidence to support this contention. Cocaine produces a prolonged sympathoexcitatory response in humans that is buffered by baroreflexes with considerable variability in the initial sympathetic response between individuals (Jacobsen et al., 1997; Vongpatanasin et al., 1999). Therefore, identifying those individuals with greater sympathetic responsiveness may be the key to determining those individuals at risk for cocaine-induced toxicity.

Acknowledgements

The authors would like to thank Vernon Fischer for analysis of histologic slides of the hearts and Erin L. Winkeler for assistance with preparing the manuscript. This work was supported by a grant from the USPHS DA05180.

References

Amin, M., Gabelman, G., Karpel, J., Buttrick, P., 1990. Acute myocardial infarction and chest pain syndromes after cocaine use. Am. J. Cardiol. 66, 1434–1437.

Branch, C.A., Knuepfer, M.M., 1992. Adrenergic mechanisms underlying cardiac and vascular responses in conscious rats. J. Pharmacol. Exp. Ther. 263, 742–751.

Branch, C.A., Knuepfer, M.M., 1994a. Causes of differential cardiovascular sensitivity to cocaine: I. Studies in conscious rats. J. Pharmacol. Exp. Ther. 269, 674–683.

Branch, C.A., Knuepfer, M.M., 1994b. Causes of differential cardiovascular sensitivity to cocaine: II. Sympathetic, metabolic and cardiac effects. J. Pharmacol. Exp. Ther. 271, 1103–1113.

Catravas, J.D., Waters, I.W., 1981. Acute cocaine intoxication in the conscious dog: studies on the mechanism of lethality. J. Pharmacol. Exp. Ther. 217, 350–356.

Derlet, R.W., Albertson, T.E., 1990. Acute cocaine toxicity: antagonism by agents interacting with adrenoceptors. Pharmacol. Biochem. Behav. 36, 225–231.

Dong, H.W., Gan, Q., Knuepfer, M.M., 2001. Central corticotropin releasing factor (CRF) and adrenergic receptors mediate hemodynamic responses to cocaine. Brain Res. 893, 1–10.

Flores, E.D., Lange, R.A., Cigarroa, R.G., Hillis, L.D., 1990. Effect of cocaine on coronary artery dimensions in atherosclerotic coronary artery disease: enhanced vasoconstriction at sites of significant stenoses. J. Am. Coll. Cardiol. 16, 74–79.

Gay, G.R., 1982. Clinical management of acute and chronic cocaine poisoning. Ann. Emerg. Med. 11, 562–572.

George, F., 1991. Cocaine toxicity: genetic evidence suggests different mechanisms for cocaine induced seizures and lethality. Psychopharmacology 104, 307–311.

Guinn, M.M., Bedford, J.A., Wilson, M.C., 1980. Antagonism of intravenous cocaine lethality in nonhuman primates. Clin. Toxicol. 16, 499–508.

Heavner, J.E., Shi, B., Pitkänen, M., 1995. Effects of nitric oxide inhalation on responses to systemic cocaine administration in rats. Life Sci. 57, 715–728.

Isner, J.M., Estes, N.A.M., Thompson, P.D., Costanzo-Nordin, M.R., Subramanian, R., Miller, G., Katsas, G., Sweeney, K., Sturner, W.Q., 1986. Acute cardiac events temporally related to cocaine abuse. N. Engl. J. Med. 315, 1438–1443.

Jacobsen, T.N., Grayburn, P.A., Snyder, R.W., Hansen, J., Chavosen, B., Landau, C., Lange, R.A., Hillis, L.D., Victor, R.G., 1997. Effects of intranasal cocaine on sympathetic nerve discharge in humans. J. Clin. Invest. 99, 628–634.

Karch, S.B., Stephens, B., Ho, C.H., 1998. Relating cocaine blood concentrations to toxicity—an autopsy study of 99 cases. J. Forensic Sci. 43, 41–45.

Knuepfer, M.M., Branch, C.A., 1992. Cardiovascular responses to cocaine are initially mediated by the central nervous system in rats. J. Pharmacol. Exp. Ther. 263, 734–741.

Knuepfer, M.M., Mueller, P.J., 1999. Review of evidence for a novel model of cocaine-induced cardiovascular toxicity. Pharmacol. Biochem. Behav. 63, 489–500.

Knuepfer, M.M., Branch, C.A., Gan, Q., Fischer, V.W., 1993. Relationship between cocaine-induced cardiac ultrastructural alterations and cardiac output responses in rats. Exp. Mol. Pathol. 59, 155–168.

Knuepfer, M.M., Gan, Q., Mueller, P.J., 1998. Mechanisms of hemodynamic responses to cocaine in conscious rats. J. Cardiovasc. Pharmacol. 31, 391–399.

Knuepfer, M.M., Purcell, R.M., Gan, Q., Le, K.M., 2001. Hemodynamic response patterns to acute behavioral stressors resemble those to cocaine. Am. J. Physiol., Regul. Integr. Comp. Physiol. 281, R1778–R1786.

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

Lange, R.A., Cigarroa, R.G., Flores, E.D., Flores, E.D., McBride, W., Kim, A.S., Wells, P.J., Bedotto, J.B., Danziger, R.S., Hillis, L.D., 1990. Potentiation of cocaine-induced coronary vasoconstriction by beta-adrenergic blockade. Ann. Int. Med. 12, 897–903.

Mets, B., Jamdar, S., Landry, D., 1996. The role of catecholamines in cocaine toxicity: a model for cocaine "sudden death". Life Sci. 59, 2021–2031.

Minor Jr., R.L., Scott, B.D., Brown, D.D., Winniford, M.D., 1991. Cocaine-induced myocardial infarction in patients with normal coronary arteries. Ann. Int. Med. 115, 797–806.

Mittleman, R.E., Wetli, C.V., 1987. Cocaine and sudden "natural" death. J. Forensic Sci. 32, 11–19.

Mueller, P.J., Gan, Q., Knuepfer, M.M., 1997. Ethanol alters hemodynamic responses to cocaine in conscious rats. Drug Alcohol Depend. 48, 17–24.

Muller, J.R., Le, K.M., Haines, W.R., Gan, Q., Knuepfer, M.M., 2001. Hemodynamic response pattern predicts susceptibility to stress-induced elevation in arterial pressure in the rat. Am. J. Physiol., Regul. Integr. Comp. Physiol. 281, R31–R37.

Poon, J., van den Buuse, M., 1998. Autonomic mechanisms in the acute cardiovascular effects of cocaine in conscious rats. Eur. J. Pharmacol. 363, 147–152.

Purcell, R.M., Gan, Q., Knuepfer, M.M., 2001. Variable renal sympathetic responses to cocaine in rats. FASEB J. 15, 801 (abstract).

Ramoska, E., Sacchetti, A.D., 1985. Propranolol-induced hypertension in treatment of cocaine intoxication. Ann. Emerg. Med. 14, 1112–1113.

Rappolt, R.T., Gay, G.R., Inaba, D.S., 1977. Propranolol: a specific antagonist to cocaine. Clin. Toxicol. 10, 265–271.

Ruttenber, A.J., Lawler-Heavner, J., Ying, M., Wetli, C.V., Hearn, W.L., Mash, D.C., 1997. Fatal excited delirium following cocaine use: epidemiologic findings provide new evidence for mechanisms of cocaine toxicity. J. Forensic Sci. 42, 25–31.

Shi, B., Heavner, J.E., Liu, J., Wang, M.J., Lutherer, L.O., McIntyre, D.C., Reigel, C.E., 1999. Two genetically selected strains of rats exhibit hypersensitivity or resistance to cocaine-induced fatal arrhythmias. J. Pharmacol. Exp. Ther. 288, 685–692.

Smart, R.G., Anglin, L., 1987. Do we know the lethal dose of cocaine? J. Forensic Sci. 32, 303–312.

Smith, M., Garner, D., Niemann, J.T., 1991. Pharmacologic interventions after an LD50 cocaine insult in a chronically instrumented rat model: are beta-blockers contraindicated? Lab. Invest. 20, 768–771.

Tella, S.R., Schindler, C.W., Goldberg, S.R., 1992. Pathophysiological and pharmacological mechanisms of acute cocaine toxicity in conscious rats. J. Pharmacol. Exp. Ther. 262, 936–946.

Trouvé, R., Nahas, G.G., 1990. Antidotes to lethal cocaine toxicity in the rat. Arch. Int. Pharmacodyn. 305, 197–207.

Vongpatanasin, W., Mansour, Y., Chavoshan, B., Arbique, D., Victor, R.G., 1999. Cocaine stimulates the human cardiovascular system via a central mechanism of action. Circulation 100, 497–502.

Wetli, C.V., Wright, R.K., 1979. Death caused by recreational cocaine use. J. Am. Med. Assoc. 241, 2519–2522.

Wilson, M.C., Holbrook, J.M., 1978. Intravenous cocaine lethality in the rat. Pharmacol. Res. Commun. 10, 243–256.

Witkin, J.M., Goldberg, S.R., Katz, J.L., Kuhar, M.J., 1989. Modulation of the lethal effects of cocaine by cholinomimetics. Life Sci. 45, 2295–2301.