Cardiovascular Responses Elicited by the “Binge” Administration of Methamphetamine

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ABSTRACT

Methamphetamine (METH) abuse is often characterized by a repeated pattern of frequent drug administrations (binge) followed by a period of abstinence. The effect of this pattern of METH use on cardiovascular function has not been characterized. Radiotelemetry was used to record the cardiovascular responses elicited during three successive METH binges (3 mg/kg, b.i.d. for 4 days) in conscious rats. Each binge was followed by a 10-day METH-free period. The effects of METH administration on vascular reactivity, Bezold-Jarisch reflex function, and cardiac morphology were also evaluated. The pressor responses elicited by the first three doses of METH in the second and third binges were significantly larger than those elicited by the corresponding doses in the first binge. The heart rate (HR) responses elicited by METH were similar within and among the three binges. Ten days after the last binge, the depressor responses elicited by the i.v. injection of sodium nitroprusside, isoproterenol, and acetylcholine were significantly smaller than those elicited before each binge. The arterial pressure and HR responses elicited by phenylephrine were unchanged. Bezold-Jarisch reflex function evoked by i.v. serotonin (10 μg/kg) was significantly altered. The hearts from treated rats showed focal inflammatory infiltrates with abundant monocytes and occasional necrotic foci. These results indicate that this binge pattern of METH administration can significantly alter cardiovascular function and cardiovascular reflex function and produce serious cardiac pathology.

The illicit use of methamphetamine (METH) has dramatically increased during the past several years, as has the number of clinical reports detailing serious and sometimes fatal cardiovascular toxicity associated with the use of this drug (Hong et al., 1991; Karch et al., 1999). Despite the potential dangers posed by METH, the cardiovascular effects of this drug have not been well characterized. Most experimental and clinical studies examining the cardiovascular actions of METH have focused on the acute arterial pressure (AP) and heart rate (HR) effects of the drug (Martin et al., 1971; Schindler et al., 1992). However, METH abuse typically involves a pattern of repeated drug administration. Only a handful of studies have looked at the cardiovascular responses elicited by repeated METH administration. For example, in humans given daily oral doses of METH, tolerance develops to the tachycardic but not the pressor actions of the drug (Perez-Reyes et al., 1991). In hypertensive dogs, daily oral administration of METH lowers AP but does not alter the magnitude of the pressor response elicited by METH (Vidrio, 1982). Conversely, in normotensive dogs the magnitude of the pressor response, but not the baseline AP, is lowered by the same dosing regime (Vidrio, 1982). In rats given single, intermittent i.p. doses of METH (six doses spaced 3–4 days apart), there is sensitization to the pressor effects of METH; however, the tachycardic responses and baseline cardiovascular parameters are not altered (Yoshida et al., 1993).

A common pattern of METH abuse is characterized by a pattern of frequent (several times a day) drug administration for a short period (“run” or “binge”) followed by a drug-free period (Konuma, 1994). This cyclic pattern of frequent, short-term use and abstinence is usually repeated many times. The cardiovascular responses elicited by this pattern of METH abuse have not been examined. This information is of clinical significance given the possibility that sensitization to the cardiovascular actions of METH responses may develop during this pattern of intermittent drug administration. Sensitization of the behavioral and body temperature responses during the intermittent administration of amphetamine-like compounds has been well documented (see Robinson and Becker, 1986). Therefore, one goal of this study was to test...
the hypothesis that sensitization of the AP and HR responses elicited by the i.v. administration of METH would develop during several cycles of binge and abstinence. To minimize the potential cardiovascular effects produced by the stress of handling the animals and the long duration of the protocol, these studies were conducted using a radiotelemetry recording system. Drugs were administered by the i.v. route to approximate more closely the rapid increase in the blood levels of METH produced in individuals who inject or smoke the drug.

We recently reported that repeated administration of neurotoxic doses of the amphetamine analog, 3,4-methylenedioxymethamphetamine, significantly enhanced the bradycardic component of the Bezold-Jarisch reflex elicited by i.v. serotonin (OCain et al., 2000). Therefore, a second goal of this study was to test the hypothesis that the repeated intermittent administration of METH would also enhance this cardiovascular reflex response. In addition, we tested the hypothesis that this pattern of METH administration would change the AP or HR responses elicited by a variety of vasoactive agents.

METH produces cardiac pathology in animals after chronic dosing (Islam et al., 1995; He et al., 1996) and is often observed in the hearts of human METH users at autopsy (Hong et al., 1991; Karch et al., 1999). Therefore, we tested the hypothesis that a binge pattern of METH administration would also produce cardiac pathology.

Experimental Procedures

General Methods. Experiments were performed using male Sprague-Dawley rats (280–340 g; Harlan, Indianapolis, IN). All procedures were in accordance with National Institutes of Health guidelines for the care and use of experimental animals and were approved by the Institutional Animal Care and Use Committee at Louisiana State University Health Sciences Center. Before surgery,
the rats were housed in groups in a temperature- and humidity-controlled room with a 12-h light/dark cycle. After surgery, the animals were housed individually in the same room. Standard rat chow and tap water were available ad libitum. During all surgical procedures, the rats were anesthetized using methohexital sodium (5–7 mg/kg, i.p.). Anesthesia was supplemented (2–3 mg/kg, i.p.) as indicated by spontaneous changes in respiration, cardiovascular parameters, and/or movement in response to tail or foot pinch.

Mean arterial pressure (MAP) and HR were measured in conscious, freely moving rats in their home cage using a radiotelemetry system (Dataquest A.R.T.; Data Sciences International, St. Paul, MN). The battery-operated telemetry probe (TL11 M2-C50-PXT) contained an arterial catheter and a pressure transducer. Under methohexital sodium anesthesia, the arterial catheter was inserted into the descending aorta just rostral to the femoral bifurcation. The telemetry probe was then placed in the abdominal cavity and sutured to the abdominal musculature. A polyurethane venous cannula (Mircorenathane; 0.33 o.d. × 0.014 i.d.; Braintree Scientific, Braintree, MA) was placed in the femoral vein for the administration of drugs. The free end of the venous cannula was tunneled subcutaneously to the nape of the neck and exteriorized.

Data Analysis. The output from the telemetry probes (frequency in hertz) was recorded by a receiver under the home cage. The data were then sent to a consolidation matrix before being stored on a personal computer. Data acquisition was controlled using DataSciences International Dataquest acquisition software. During the experiments, MAP and HR data were continuously collected at 500 Hz. Data were then averaged into 1-s bins and displayed. The peak MAP and HR responses elicited by drug administrations were calculated using the Dataquest analysis program. Baseline MAP and HR were recorded for each animal before each injection. Mean baseline values were compared using a one-way repeated-measures analysis of variance (rmANOVA). The MAP and HR responses elicited by METH within and among binges were compared using two-way rmANOVA. The MAP and HR responses elicited by acetylcholine (Ach), phenylephrine (PE), sodium nitroprusside (NP), isoproterenol (Iso), and serotonin (5-hydroxytryptamine; 5-HT) before each binge and 10 days after the third binge were compared using one-way rmANOVA. After all ANOVAs, the differences among individual means were evaluated using Student-Newman-Keuls tests.

Experimental Protocol. Twelve rats were instrumented with the telemetry probes 7 to 10 days before the start of the experiments. The day before the experiment, baseline MAP and HR were recorded for 30 to 45 min. The rats were then given bolus doses of Ach (6 μg/kg), PE (9 μg/kg), NP (45 μg/kg), Iso (30 μg/kg), and 5-HT (10 μg/kg), and the MAP and HR responses were recorded. The cardiovascular parameters were allowed to return to control levels before administering the next test drug. In preliminary studies, these doses of Ach, NP, Iso, PE, and 5-HT were found to produce large but submaximal MAP and HR responses (data not shown). Ach, Iso, and PE were used to determine whether prolonged exposure to the sympathomimetic actions of METH would alter muscarinic and β adrenergic and/or α adrenergic receptor-mediated cardiovascular responses. NP was used to determine whether repeated exposure to METH would alter a nonreceptor-mediated vasodilatory response. NP was used to determine whether repeated exposure to METH and 3 saline-treated rats were deeply anesthetized using halothane, and the hearts were quickly removed. The isolated hearts were then perfused retrogradely through the aorta with phosphate-buffered saline followed by 10% zinc formalin. Four METH- and three saline-treated rats were similarly sacrificed, and the hearts were removed 1 day after the end of the second and third binges. The perfused hearts were cut into a basal, two midventricular, and an apical section and fixed for 4 to 6 h in 10% zinc formalin. The sections were then embedded in paraffin, sectioned (2 μm), and placed on glass slides. Alternate sections were stained with Mason’s trichrome or H&E and coverslipped.

For immunohistochemistry, additional 4-μm sections were cut and stained with a monoclonal antibody that recognizes rat tissue macrophages/monocytes and myeloid cells (ED1, MAC341R, and Statt 77, MCA341R; Serotec Inc., Raleigh, NC). As a positive control, each batch of slides was coincubated with sections of rat intestine and spleen. Negative controls consisted of tissue incubated without the primary antibody. Positive cells were identified by the brown granular pigmentation in the cytoplasm.

All histological sections were examined blind by the same pathologist. Inflammation was diagnosed on H&E slides by the accumulation of mononuclear and polymorphonuclear inflammatory cells. The presence of monocytes was confirmed by examining the adjacent sections stained with the ED1 antibody. The extent of myocardial inflammatory infiltrate was classified as either 1) an interstitial lesion when three or more inflammatory cells (exclusive of mast cells) were present in the interstitium or around blood vessels, without distruption of myocardial fibers (Fig. 6) or 2) a focal lesion when inflammatory infiltrate was associated with a disruption of the normal architecture, with or without necrosis (Fig. 7). For each rat, one H&E-stained slide from each of the four gross heart sections was examined, and the number of interstitial and focal lesions was counted. Student’s t tests were used to compare the number of interstitial and focal lesions observed in the METH- and saline-treated rats at each time point. The total number of lesions in treated and control rats at each time point was also compared using t tests.

Drugs used in this study were (+)-methamphetamine HCl, phenylephrine HCl, isoproterenol, acetylcholine, sodium nitroprusside, serotonin HCl (all from Sigma-Aldrich, St. Louis, MO), and methohexital sodium (Brevital sodium; Jones Pharma, St. Louis, MO).

Results

Table 1 summarizes the baseline levels of MAP and HR the day before each of the three METH binges and 10 days after

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Before Binge 1</th>
<th>Before Binge 2</th>
<th>Before Binge 3</th>
<th>After Binge 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>112 ± 4</td>
<td>117 ± 3</td>
<td>117 ± 3</td>
<td>117 ± 3</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>400 ± 10</td>
<td>376 ± 10**</td>
<td>369 ± 9**</td>
<td>365 ± 13*</td>
</tr>
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*p < 0.05 and **p < 0.01 indicate that the mean is significantly different from that before the start of the first methamphetamine binge.
the third binge. There were no significant differences in the baseline levels of MAP at any of these time points. However, baseline HR rate was significantly higher before binge 1 than before either the second or third binges or 10 days after the third binge.

The i.v. administration of METH (3 mg/kg) elicited a pressor response consisting of an initial rapid increase in MAP lasting a few seconds, followed by a more prolonged, lower amplitude increase in pressure (Fig. 1). METH elicited biphasic HR responses consisting of bradycardia followed by tachycardia (Fig. 1). Figure 2 summarizes the peak (initial portion of the response) MAP and HR responses elicited by each dose of METH within each of the three binges. Within the first binge, all eight doses of METH elicited similar pressor responses. During the course of the second binge, there was a dose-related decrease ($p < 0.0001$) in the magnitude of the pressor responses. The pressor responses elicited by the first three doses of METH in the second binge were also significantly larger than those elicited by the corresponding doses in the first binge (Fig. 2). There was a dose-related decrease ($p < 0.0001$) in the magnitude of the pressor responses within the third binge, and the pressor responses elicited by the first three doses of METH were significantly larger than those elicited by the corresponding doses in the first binge (Fig. 2). The pressor responses in the second binge were not significantly different from those of the corresponding doses in the third binge. The magnitude ($13 \pm 2 \text{ mm Hg}$) and duration ($10 \pm 2 \text{ min}$) of the secondary portion of the pressor responses were similar within or among the binges.

Although the magnitude of the bradycardic responses elicited by METH tended to decrease between the first and eighth doses within each binge, these decreases were not significant (Fig. 2). There were no significant differences in the magnitudes of the bradycardic responses to METH among the three binges. Likewise, there were no significant differences in the magnitudes of the tachycardic responses elicited by METH either within or among binges (Fig. 2).

Figure 3 summarizes the MAP and HR responses elicited by the i.v. administration of Ach, NP, and Iso 1 day before the start of the first, second, and third binges and 10 days after the third binge. Ten days after the third binge, the depressor responses elicited by NP and Iso were significantly smaller than the responses elicited before the first binge (Fig. 3). The depressor responses elicited by Ach 10 days after the third binge were significantly smaller than those elicited before the third binge (Fig. 3). There were no significant differences in the magnitudes of the HR responses elicited by Ach, NP, or Iso at any of the time points (Fig. 3). The MAP and HR responses elicited by Ach, NP, and Iso in rats given multiple injections of saline ($n = 3$) were similar across the binges and demonstrate the stability of these responses over the long duration of this study. Comparisons between the METH- and the saline-treated rats were not made because the study was designed to allow the METH-treated rats to serve as their own controls and because of the small number of saline-treated rats.

Figure 4 summarizes the MAP and HR responses elicited by the i.v. injection of PE. There were no significant differences in the MAP or HR responses elicited by PE at any of the time points tested. Before the first METH binge, the i.v. administration of 5-HT elicited hypotension and bradycardia characteristic of Bezold-Jarisch reflex activation (Fig. 5). After treatment with METH, the hypotensive responses were converted to pressor responses (Fig. 5). The HR responses elicited by 5-HT were not significantly affected by any of the METH treatments, although they were markedly reduced 10 days after the third binge. In fact, after the third binge, 5-HT elicited tachycardia ($69 \pm 8 \text{ bpm}$) in five of the rats.

Rats subjected to three METH binges showed randomly distributed myocardial lesions throughout the heart. Lesions consisted of predominantly mononuclear inflammatory infiltrate with interstitial (Fig. 6) or focal distribution (Fig. 7). Necrosis was observed in some focal lesions (Table 2, Fig. 7). The inflammatory infiltrate contained abundant monocytes and macrophages as demonstrated by immunohistochemistry. Pathologic fibrosis was not observed in any of the hearts. The presence of disseminated mast cells and contraction bands was similar in experimental and control hearts. Table 2 summarizes the number of interstitial and focal lesions observed in the METH-treated and control rats subjected to one, two, or three binges. The range of the data rather than the standard error of the mean is shown due to the relatively small number of animals in each group. The total number of lesions observed in each group at each time point is also shown. Rats subjected to three METH binges had significantly more interstitial lesions than did the corresponding control rats. Although there appeared to be an increase in the number of focal lesions in the treated rats, this increase was not statistically significant. After three binges, the total number of lesions in the treated rats was also significantly greater than in the control rats. There were no significant differences in the number of lesions observed in the treated and control rats subjected to one or two binges.

Discussion

To our knowledge, this study is the first to characterize the cardiovascular and cardiovascular reflex responses elicited within and among a series of METH binges. As expected, the i.v. administration of METH elicited pressor responses (Schindler et al., 1992). METH also elicited biphasic HR responses consisting of an initial bradycardia followed by tachycardia. Similar decreases in HR also occur after i.v. injection of METH in conscious monkeys (Schindler et al., 1992) and i.v. amphetamine in conscious rats (O’Cain et al., 2000). METH elicits tachycardia in humans (Perez-Reyes et al., 1991). The consistency of the MAP and HR responses elicited by each dose of METH during the first METH binge indicated that tachyphylaxis to these responses did not develop during the twice-daily administration of the drug. Tachyphylaxis to the pressor effects of orally administered METH develops in normotensive but not renal hypertensive dogs (Vidrio, 1982). Tachyphylaxis to the tachycardic effects of METH also develops during daily oral administration in humans (Perez-Reyes et al., 1991). Whether or not the lack of tachyphylaxis in our rats reflects differences in the route of administration or species differences is unknown.

In the second and third binges, the pressor responses elicited by the first two or three doses of METH were significantly larger than those elicited by the corresponding dose in the first binge. Yoshida and colleagues (1993) previously reported that sensitization to the pressor and hyperthermic effects of METH develops in rats after intermittent injection.
(six single doses separated by 3–4 days). The time course of the increase in sensitivity is unknown because only the responses elicited by the first and sixth doses were reported. The increased sensitivity to the pressor effects of METH following periods of abstinence is reminiscent of the behavioral sensitization produced by the intermittent administration of METH and other amphetamine-like drugs (Robinson and Becker, 1986). The question of whether sensitization to the cardiovascular actions of METH also occurs in humans after intermittent administration may have important clinical implications in terms of the toxicity of this drug.

To determine whether repeated exposure to the sympathomimetic actions of METH altered vascular reactivity, the MAP and HR responses elicited by a series of pressor and depressor agents were recorded before each binge and 10 days after the third binge. The MAP and HR responses elicited by these drugs in rats treated with saline (n = 3) are also shown. Values are means ± S.E.M. *, p < 0.05 and **, p < 0.01 significantly different from response before the first binge.

**Fig. 3.** Summary of the peak MAP and HR responses (n = 9) elicited by the i.v. injection of isoproterenol (30 µg/kg), acetylcholine (6 µg/kg), and sodium nitroprusside (45 µg/kg) before each METH binge and 10 days after the third binge. The MAP and HR responses elicited by these drugs in rats treated with saline (n = 3) are also shown. Values are means ± S.E.M. *, p < 0.05 and **, p < 0.01 significantly different from response before the first binge.
Although not different before each of the binges, the depressor responses elicited by NP, Ach, and Iso were significantly reduced 10 days after the third binge. The mechanisms responsible for the decreased responsiveness of the vasculature to the vasodilator actions of these agents are not clear. Vascular relaxation produced by Ach and NP involves nitric oxide, guanylate cyclase production, and the regulation of intracellular calcium to produce relaxation (Ignaro, 1981). In contrast, Iso relaxes vascular smooth muscle via the activation of $\beta_2$-adrenergic receptors, cAMP production, and the regulation of protein kinases (Katzung and Chatterjee, 1982). It is possible that METH alters both pathways by different mechanisms. Alternatively, the decrease in vasodilator action may reflect vascular remodeling resulting from the METH-induced increases in AP and/or the release of a
hypertrophic factor. Vascular remodeling may explain why the decreased responsiveness to these agents does not occur until after the third binge, 48 days after the first dose of METH. Arguing against a role for vascular remodeling are the observations that the baseline MAP was not increased after each binge and that the vasoconstrictor responses elicited by PE were similar before and after each binge.

Although the depressor responses elicited by NP were significantly reduced 10 days after the third binge, the HR responses remained unchanged. Because the tachycardia elicited by NP is reflex in nature, it appears that treatment with METH changed baroreceptor sensitivity. Previous studies have speculated that METH alters baroreceptor reflex function by a direct action on the vasculature around the carotid sinus (Heymans, 1955). We attempted to quantify baroreceptor function by measuring the HR changes elicited during ramped increases and decreases in MAP. However, due to difficulties in monitoring and regulating the changes in MAP using the telemetry system, these studies were unsuccessful.

Before the first binge, the i.v. injection of serotonin elicited hypotension and bradycardia typical of the vasovagal Bezold-Jarisch reflex (Thoren, 1979; Verberne and Guyenet, 1992). After treatment with METH, the hypotensive response was converted to a pressor response. The bradycardic portion of the response was more stable, but after the third binge, it was nearly eliminated. Whether these changes reflected a rightward shift in the dose-response relationship for 5-HT and HR needs to be tested. The altered responses to 5-HT may also reflect structural or functional changes in the central or peripheral portions of this vasovagal reflex arc. The repeated administration of METH produces neurotoxicity in several species (Seiden and Ricaurte, 1987; Ricaurte and McCann, 1992). Whether the doses and dosing schedule used in this study produce neurotoxicity was not tested. We recently showed that the administration of neurotoxic doses of 3,4-methylenedioxymethamphetamine (20 mg/kg, b.i.d. for 4 days), a reportedly selective serotonergic neurotoxin, enhanced the bradycardic portion of the Bezold-Jarisch reflex for up to 2 weeks after administering the last dose (Commins et al., 1987; O’Cain et al., 2000). The fact that these two structurally similar amphetamine derivatives produced opposite effects on Bezold-Jarisch reflex function after repeated administration may reflect differences in the spectrum of neurotoxicity (one broad and one selective) produced by these drugs. This possibility is currently being studied.

The physiological role of the Bezold-Jarisch reflex is largely unknown. The chemosensitive portion of this reflex can be activated in response to cardiac ischemia, hypoxia, or changes in preload (Coleridge and Coleridge, 1980). The mechanosensitive elements of the Bezold-Jarisch reflex respond to changes in atrial and ventricular wall stress and may contribute to neurocardiogenic and vasovagal syncope (Somers and Mark, 1996). Because METH can increase inotropy and produce cardiac ischemia, changes in Bezold-Jarisch reflex function may attenuate the ability of the animal to respond to the cardiac stress produced by this drug.

To our knowledge, this is the first study to demonstrate that the binge administration of METH can produce significant cardiac pathology. In rats subjected to this dosing regimen, we observed myocardial foci of predominantly mononuclear inflammatory infiltrates (primarily monocytes/macrophages) with areas of disrupted architecture and occasional myofibril necrosis. Mast cells, normally present in the rat myocardium (Majeed, 1994), were not increased in rats receiving METH. Similar types of cardiac toxicity have been reported in animals after acute and chronic administration of METH (Islam et al., 1995; He et al., 1996). A growing clinical literature has also linked METH use/abuse with cardiac toxicity and death in humans (Hong et al., 1991; Chan et al., 1994; Karch et al., 1999). However, the toxic effects of METH in these clinical reports are often difficult to evaluate due to the polydrug use and a lack of data regarding quantity and frequency of METH administration.

Although several potential mechanisms have been proposed, the mechanisms responsible for the cardiac toxic actions of METH are unknown. One potential mechanism suggests that a METH-induced increase in peripheral catecholamines is responsible for the cardiotoxicity (see Jiang and Downing, 1990). It is known that catecholaminergic stimulation can produce myocardial necrosis and infarction similar to that observed after administering METH (Downing and Chen 1985; Simons and Downing, 1985; Jiang and Downing, 1990). As reviewed by Jiang and Downing (1990), the mechanisms mediating catecholamine-induced cardiac damage may include ischemia due to catecholamine-mediated coronary vasoconstriction, calcium overload, and the production of oxygen free radicals by either the autooxidation of catecholamines or their degradation by monoamine oxidase. Reactive oxygen species may also be produced by catecholamine degradation, mitochondrial dysfunction, leukocyte activation, and/or xanthine oxidization during the reperfusion of ischemic areas.

Alternatively, METH may produce cardiac damage by direct effects on the myocytes. METH is cytotoxic to myocytes in culture systems devoid of catecholamines (Welder, 1992;
He, 1995); however, the mechanisms responsible for these toxic effects are unknown. METH may also damage cardiac cells by initiating apoptosis. Apoptotic processes occur in several pathological conditions including myocardial ischemia and reperfusion, infarction, and cardiomyopathy (Song et al., 1999; Webster et al., 1999; Oskarsson et al., 2000; Xie et al., 2000). Whether or not apoptosis is involved in METH-induced cardiac toxicity is being tested.

The binge administration of METH can produce significant changes in cardiovascular and cardiovascular reflex function and result in significant cardiac pathologic. During the binge administration of METH, there was an increase in sensitivity to the pressor actions of the drug and decreases in sensitivity to the depressor actions of NP, ISO, and Ach. Zebsd-Jarisch reflex function elicited by 5-HT, an index of cardiovascular reflex function, was also significantly altered after treatment with METH. Finally, binge administration of METH produced focal monocyte inflammatory infiltrates and foci of necrosis in the hearts of treated rats. These results underscore the potential for this commonly used pattern of METH administration to significantly alter cardiovascular function and produce cardiac pathology.

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References