Differential Depressant Effects of General Anesthetics on the Cardiovascular Response to Cocaine in Mice (44411)

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Abstract. **In recent years, murine models have gained increasing importance for studies of cardiovascular physiology and pharmacology, largely due to the development of transgenic strains with specific alterations in phenotype. Differential effects of general anesthetic agents on the cardiovascular responses to cocaine have been reported in larger mammals; therefore, we studied the effects of commonly used anesthetics on heart function and on blood pressure responses to cocaine in Swiss Webster mice. We positioned a polyethylene catheter (PE-10) in the right carotid artery or left ventricle of mice anesthetized with equivalent anesthetic dose of either ketamine-xylazine (KX, 40 mg/kg + 5 mg/kg), pentobarbital (PEN, 40 mg/kg) or α-chloralose-urethane (CU, 80 mg/kg + 100 mg/kg). Cocaine (0.3 mg/kg, 1 mg/kg and 3 mg/kg) was administrated *via* the left jugular vein by bolus injection. In the KX group, the basal mean arterial pressure (MAP) and systolic left ventricular pressure (LVP) were 110 ± 12 and 120 ± 13 mmHg, respectively, close to conscious values. However, PEN and CU significantly decreased the basal parameters ($P < 0.01$ compared to the KX group). The lowest dose of cocaine (0.3 mg/kg) elicited minimal changes. Significant responses were obtained with a 1-mg/kg dose of cocaine ($P < 0.01$ compared to baseline). However, at 3 mg/kg, a toxic effect of cocaine appeared in all three anesthetic groups. Compared to published conscious animal data, anesthetic agents attenuated the cardiovascular effects of cocaine. Taken together, our results indicate that minimally effective doses of general anesthetics may significantly alter the basal hemodynamic state and the responses to sympathomimetic agents in the murine model, as has been reported in larger mammalian species. We concluded that anesthesia with ketamine-xylazine provides baseline hemodynamic values close to reported values in conscious animals, but also attenuates the hemodynamic response to cocaine.** [P.S.E.B.M. 1999, Vol 221]

In recent years, there has been a dramatic increase in the number of patients with a history of cocaine abuse who demonstrate various cardiovascular signs and symptoms, such as acute myocardial ischemia and infarction, arrhythmia and sudden death, contraction band necrosis, myocarditis, cardiomyopathy, and hypertension (1–3). Moreover, long-term exposure to cocaine has been shown to induce uterine and fetal blood flow disorders, fetal growth restriction, and hypoxia (4). Studies have reported a significant influence of cocaine administration on cardiovascular function in pigs, dogs, ferrets and rats (5–7). Nunez and Morgan (8, 9) reported that acute or chronic cocaine exposure induced microvascular vasoconstriction and produced myocardial ischemia and infarction. Stambler *et al.* (10) reported that, in conscious dogs, cocaine showed a biphasic effect on blood pressure, first causing transient depression followed by enhanced left ventricular function. The changes in cardiovascular function caused by cocaine were associated with catecholamine release, and changes in cellular

This study was partially supported by NIH R01 DA 11762-01 to J.P.M.

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Received October 6, 1998. [P.S.E.B.M. 1999, Vol 221]
Accepted March 4, 1999.

0037-9727/99/2213-0253$14.00/0

calcium, cyclic GMP and cholinergic modulation (1, 11–13). Moreover, cocaine has been reported in animal studies to produce immunosuppression and inhibit lymphocyte proliferation and macrophage function (14, 15).

The ability to use the intact mouse to assess integrated cardiovascular function under both basal conditions and during infusion or bolus injection of chemical agents provides a valuable experimental tool (16–19). The mouse model may be particularly important with regard to understanding the actions of cocaine, which simultaneously affects multiple sites both centrally and peripherally. However, one problem that may potentially interfere with the collection of hemodynamic data is general anesthesia. General anesthetics are known to attenuate the cardiovascular response to cocaine in a variety of large animal models (20–22). It is reasonable to assume that the same is true in mice, although few reports of differential general anesthetic effects appear in the literature. The purpose of the present study was to evaluate the differential effect of general anesthetic agents on the cardiovascular responses to cocaine in mice to clarify: 1) whether general anesthetics affect the cardiovascular responses to cocaine in the mouse model; 2) to study the variability in cardiovascular responsiveness to different anesthetics in the murine model; and 3) to determine which anesthetic yields data close to those reported in conscious mice. In animal studies, the most common general anesthetics used were ketamine, barbiturates and α-chloralose. However, certain types of anesthetic agents are known to influence cardiovascular function and skeletal muscle contractility (23–27). If cardiovascular function is to be studied, then it is important to select an anesthetic agent that has minimal effects on hemodynamic parameters. In the present study we chose three general anesthetics: (ketamine-xylazine, pentobarbital, α-chloralose-urethane) to investigate the irrelative effects on cardiovascular responses to cocaine in Swiss Webster mice, a commonly used experimental strain.

Materials and Methods

Animals. Male Swiss Webster mice (Taconic, NY) weighing between 25 and 30 g were divided into three anesthetic groups: ketamine – xylazine (KX, 40 mg/kg + 5 mg/kg; ketamine, Abbott Laboratories, Chicago, IL; xylazine, Loyo Laboratories, Shenandoah, IA); pentobarbital (PEN, 40 mg/kg, Veterinary Laboratories, Inc., Lenexa, KS), and α-chloralose – urethane (CU, 80 mg/kg + 100 mg/kg, Sigma Chemical Co., St. Louis, MO). Anesthetics were administrated by intraperitoneal injection. These doses of anesthetics were the necessary minimal amount as judged by responses to corneal or pin-prick stimulation, and rate and depth of respiration. Supplemental dosage was infrequently required to maintain stable levels of anesthesia during the protocols.

All animal experiments were performed in accordance with National Institute of Health guidelines. Protocols were approved by the Animal Care and Use Committee of Beth Israel Deaconess Medical Center and Harvard Medical School.

Measurements of Hemodynamic Parameters. Mice were placed on a thermally controlled-operating plank in a supine position. A 4-0 silk was placed behind the front lower incisors and pulled taut to slightly extend the neck. Polyethylene tubing (PE-90, one end was beveled at 45° angle) was inserted into the trachea to maintain breathing. The left jugular vein was isolated and cannulated with PE-10 tubing for administration of cocaine or control saline. The right carotid artery was cannulated with the same sized PE tubing and was connected to a Statham P23Db pressure transducer (Gould, Cleveland, OH) to measure blood pressure.

In a second series of experiments, the tubing was carefully inserted into the right carotid artery through the ascending aorta into the left ventricle for cardiac measurements. The pressure signal from the transducer was amplified with a Gould amplifier model 2107-4490-00 (Cleveland, OH). Analog signals were digitized using a Data Translation Series (Model DI-220) analog-digital converter (Data Instruments Inc., Akron, OH), then stored and analyzed on a Windaq data-acquisition system.

Experimental Protocols. To evaluate the relative depressant effects of general anesthetics on blood pressure and heart function, cardiovascular responses to cocaine were determined in three different anesthetic groups. After 10–15 min of stabilization, cocaine was given as a bolus injection *via* the left jugular vein at doses of 0.3 mg/kg, 1 mg/kg, and 3 mg/kg. The volume of each cocaine bolus was 0.1 ml, and injection time was 30–40 sec. Arterial blood pressure or left ventricular pressure (LVP) was continuously recorded 10 min after administration of each dose of cocaine. Animals were then allowed to recover for 10–20 min before the next dose. In the KX anesthetized mice, a group of mice was given 0.1 ml saline as a vehicle control. In PEN and CU groups, 0.1 ml saline was given also (data not shown as there were no differences among the three groups). A third group was used as a control to observe the time course for stabilization of cardiovascular function during the experimental protocol.

Data Analysis. All data are presented as mean ± SEM. Differences before and after cocaine administration were tested using a paired t test. Overall statistical significance for differences among different anesthetics groups was tested by ANOVA. A value of < 0.05 was considered significant.

Results

Basal Hemodynamic Parameters. The effects of various anesthetics on basal hemodynamic parameters are shown in Table I. Importantly, there were significant differences among the anesthetic groups in hemodynamic parameters ($P < 0.05$). In the KX group, mean arterial pressure (MAP) was ≈ 30 mmHg higher than recorded in the CU and PEN groups. But, the effects of CU and PEN on basic

Table I. Basal Hemodynamic Parameters in Mice Anesthetized with Different Anesthetic Agents

	Ketamine-xylazine	α-Chloralose-urethane	Pentobarbital
N	10	8	9
HR(bpm)	299 ± 37	398 ± 15***	370 ± 50***
MAP(mmHg)	110 ± 15	76 ± 8***	78 ± 9***

Note. HR: heart rate; MAP: mean arterial blood pressure; ***P < 0.001 compared to ketamine-xylzaine group.

hemodynamic parameters were not significantly different. All three anesthetics caused a significant decrease in heart rate (HR). The HR in conscious Swiss Webster mice has been reported in the literature as 510 ± 11 (beats/min) (18). Under general anesthesia, KX caused a 41% decrease in HR. CU and PEN produced 21% and 27% decreases, respectively. There were significant differences among the KX, CU, and PEN groups in HR ($P < 0.05$). However, there were no obvious differences in respiratory rates, (114 ± 26 beats/min in KX mice, and 94 ± 9 or 98 ± 9 beats/min in CU and PEN mice, respectively).

Hemodynamic responses to cocaine. At baseline, before cocaine administration, MAP in KX-anesthetized mice was 110 ± 12 mmHg. Exposure of the animals to cocaine increased MAP in a dose-dependent manner (Fig. 1). One minute after a low-dose 0.3 mg/kg cocaine administration, MAP increased by 7%. However, cocaine (1 mg/kg) did not affect blood pressure at this time point. The maximal effect of cocaine was reached at the high dose of 3 mg/kg. Low-dose cocaine maintained a 7% increase in MAP after 3 min. Peak MAP was reached with 1 mg/kg cocaine and was maintained at the same level for several minutes ($P < 0.01$ vs. baseline). However, cardiovascular toxicity appeared after 1 min of cocaine administration at a high dose of 3 mg/kg.

In CU-anesthetized mice, changes in MAP caused by cocaine were similar to those in KX mice. After 1 min of administration, 0.3 mg/kg cocaine significantly increased MAP ($P < 0.05$ vs. baseline), whereas 1 mg/kg cocaine did not affect MAP at the same time point. However, at 3 min, 1 mg/kg cocaine caused a marked (18%) increase in MAP ($P < 0.01$ vs. baseline). The cardiovascular response to cocaine in PEN-anesthetized mice was similar to the KX and CU groups. We also observed the effect of vehicle (saline) on hemodynamics. In the saline control group, mice were given 0.1 ml saline (i.v., bolus), resulting in no significant changes in blood pressure over 10 min following injection (Fig. 2).

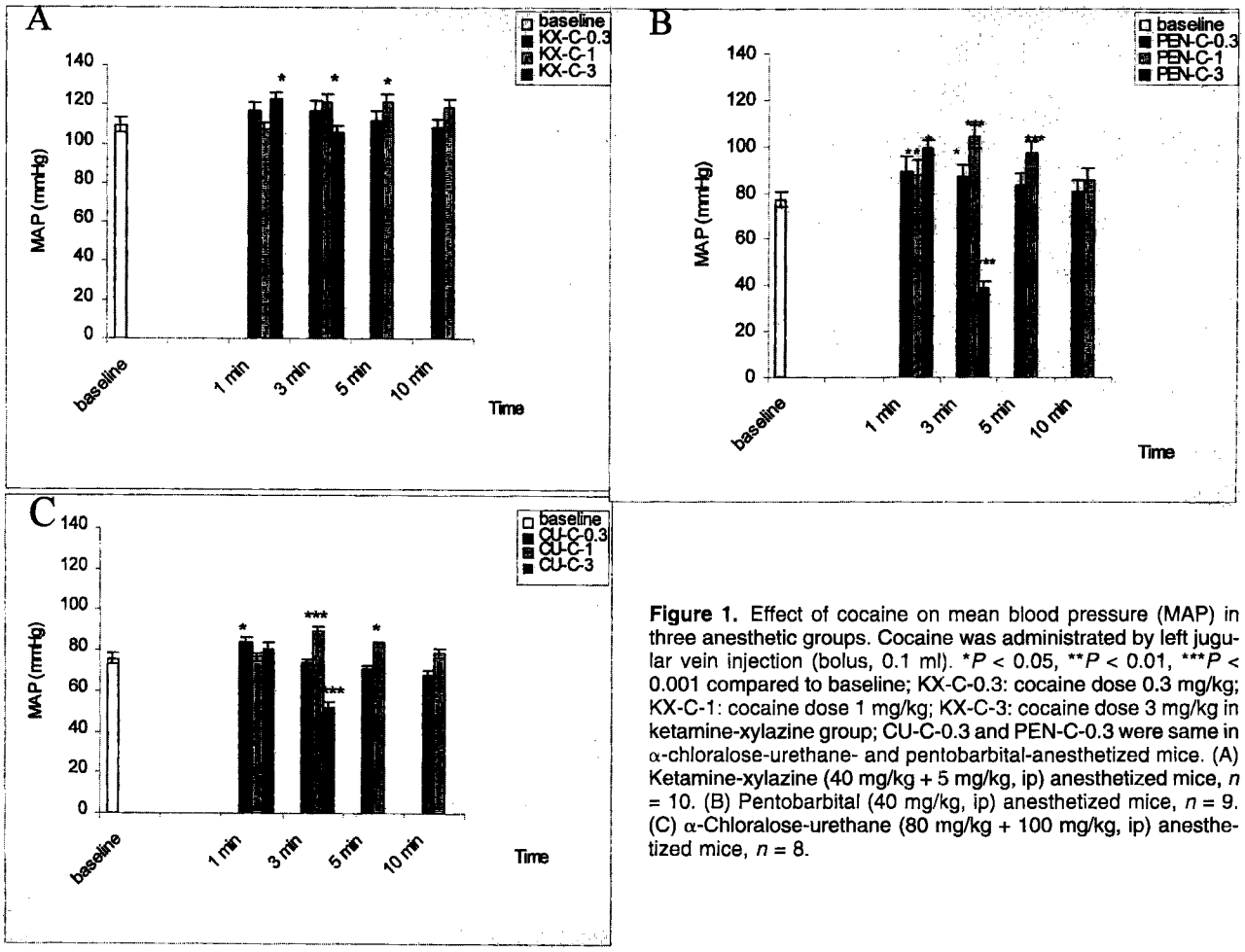

Figure 1. Effect of cocaine on mean blood pressure (MAP) in three anesthetic groups. Cocaine was administrated by left jugular vein injection (bolus, 0.1 ml). *P < 0.05, **P < 0.01, ***P < 0.001 compared to baseline; KX-C-0.3: cocaine dose 0.3 mg/kg; KX-C-1: cocaine dose 1 mg/kg; KX-C-3: cocaine dose 3 mg/kg in ketamine-xylazine group; CU-C-0.3 and PEN-C-0.3 were same in α-chloralose-urethane- and pentobarbital-anesthetized mice. (A) Ketamine-xylazine (40 mg/kg + 5 mg/kg, ip) anesthetized mice, n = 10. (B) Pentobarbital (40 mg/kg, ip) anesthetized mice, n = 9. (C) α-Chloralose-urethane (80 mg/kg + 100 mg/kg, ip) anesthetized mice, n = 8.

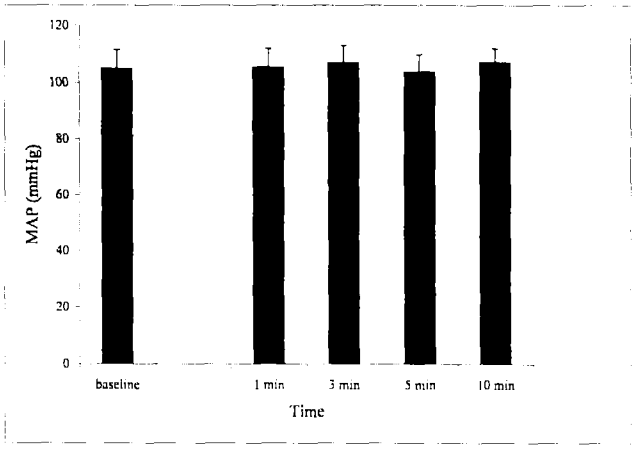

Figure 2. Effect of vehicle control on blood pressure in ketamine-xylazine (40 mg/kg + 5 mg/kg, ip) anesthetized mice. 0.1 ml saline was administrated *via* left jugular vein. $n = 7$.

Figure 3 shows the dose-response curve of blood pressure after 3 min of cocaine administration. Cocaine administration (0.3, 1, and 3 mg/kg) elicited a dose-related pressure change that was associated with increases in MAP. Overall, the lowest cocaine dose administered (0.3 mg/kg) elicited minimal changes. Significant responses were obtained with 1 mg/kg cocaine ($P < 0.01$ compared to baseline). However, the toxic effect of cocaine appeared in all three anesthetic groups with the 3 mg/kg dose, and mice died from cardiovascular system depression.

The ventricular function response to cocaine was investigated further in KX-anesthetized mice at 1 mg/kg, which had caused a significant increase in MAP in the blood pressure study. After administration of cocaine, systolic LVP was depressed and then elevated after 3 min, but was not significantly different from the pre-drug level. LVEDP increased from baseline 6 ± 1 to 15 ± 2, after 3 min ($P < 0.001$) (Fig. 4). However, +dP/dt and −dP/dt were both

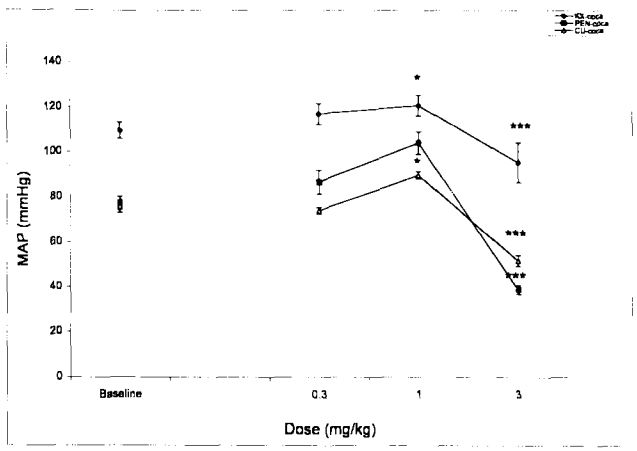

Figure 3. Dose-response curve of cocaine on mean arterial pressure in three anesthetic groups. Blood pressure was analyzed after 3 min of cocaine administration. $n = 10$ in ketamine-xylazine mice; $n = 9$ in pentobarbital group; $n = 8$ in α-chloralose-urethane mice. $^*P < 0.05$, $^{***}P < 0.001$ compared to baseline.

decreased after cocaine exposure. The time-response curve of ventricular function did not coincide with the curve of blood pressure. In the blood pressure curve, MAP was significantly increased after 3 or 5 min of cocaine administration. In the ventricular study, systolic LVP was not markedly changed, LVEDP increased, and +dP/dt decreased after 3 or 5 min of cocaine administration ($P < 0.01$).

Run-down of blood pressure in mouse model. We monitored the spontaneous decline of blood pressure in KX-anesthetized mice not exposed to additional manipulation or drugs during the period of observation. After 30 min of vessel catheterization, blood pressure did not show a significant decline. After 60 min, MAP decreased by 8%. However, after 90 min of catheterization, MAP significantly decreased ($P < 0.01$ compared to baseline) (Fig. 5). All experiments in our study were completed within 1 hr.

Discussion

In the present study, we observed the relative depressant effects of anesthetic agents on the cardiovascular response to cocaine. The salient findings are summarized as follows: 1) This study is the first to systematically evaluate *in vivo* hemodynamic parameters of mice exposed to cocaine with various anesthetic agents; 2) anesthetic agents had a depressant effect on the cardiovascular response to cocaine; 3) we concluded that anesthesia with ketamine-xylazine provides baseline hemodynamic values close to reported values in conscious animals, but also attenuates the hemodynamic response to cocaine.

Based on the literature, we initially chose the following doses of anesthetics: KX (35 mg/kg + 5 mg/kg), PEN (35 mg/kg), and CU (100 mg/kg + 100 mg/kg). However, we found that anesthesia was maintained for less than 15 min, and additional doses of anesthetic were frequently required in the KX and PEN groups. When the doses of both ketamine and pentobarbital were increased to 40 mg/kg, supplemental doses were not frequently required during the experimental period. Moreover, Konduri *et al.* (28) reported that 90 mg/kg α-chloralose decreased blood pressure from control 88 ± 11 to 74 ± 16 mmHg, but HR was not changed. However, with a dose of 60 mg/kg, systolic blood pressure increased from 88 to 100 mmHg, and HR increased about 30%. Therefore, in our study we chose the following doses of anesthetics: KX (40 mg/kg + 5 mg/kg), PEN (40 mg/kg), and CU (80 mg/kg + 100 mg/kg).

The results of the present study suggest that PEN is not the most suitable anesthetic for study of cardiovascular function *in vivo* in the mouse model. Our experiments demonstrated PEN produced marked depression of the cardiovascular system. During anesthesia, HR and MAP were 370 ± 50 beat/min and 77 ± 9 mmHg, respectively. However, studies have reported basal HR within the range of 550–620 beat/min, and MAP at 100–115 mmHg in conscious mice (18, 19, 29). Many studies have demonstrated that general anesthetic agents reduce blood pressure, HR, and ventricu-

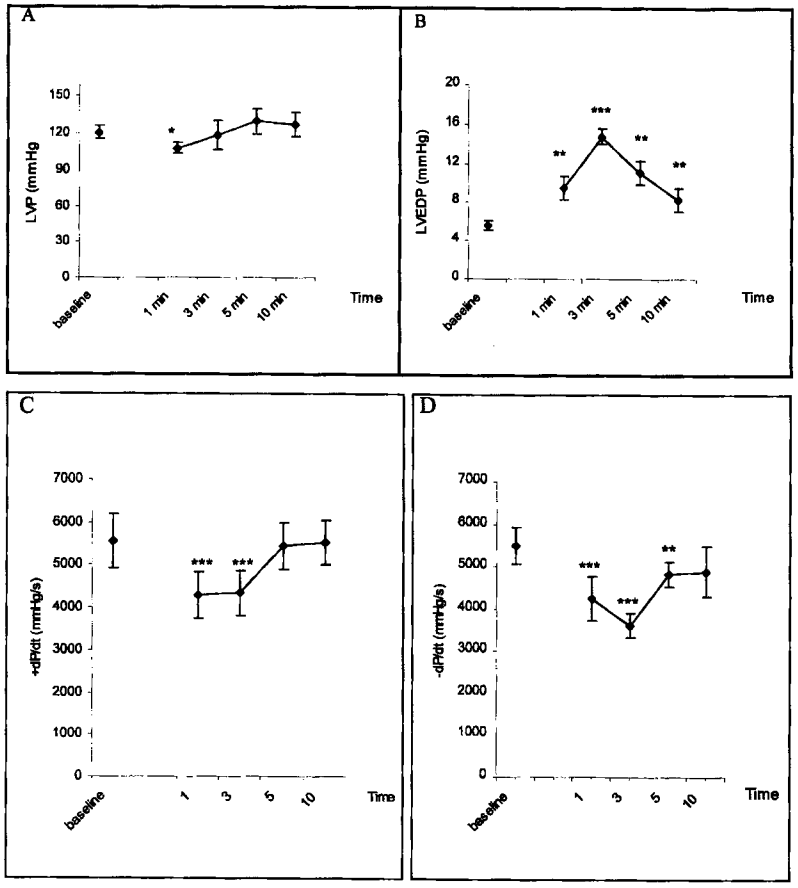

Figure 4. Effects of cocaine on mouse cardiac function. Mice were anesthetized with ketamine-xylazine (40 mg/kg + 5 mg/kg, ip), and cocaine was injected *via* left jugular vein (bolus, 1 mg/kg). $n = 6$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to baseline. (A) Effect on systolic left ventricular pressure (LVP). (B) Effect on left ventricular end-diastolic pressure. (C) Effect on +dP/dt. (D) Effect on −dP/dt.

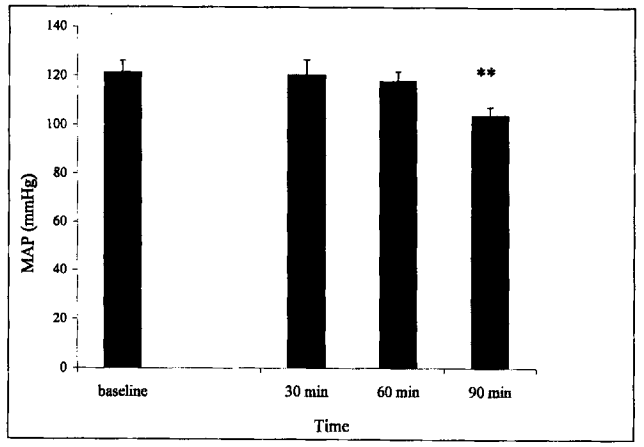

Figure 5. Time course of hemodynamic in control mouse. Mice were anesthetized with ketamine-xylazine (40 mg/kg + 5 mg/kg, ip). $n = 5$, $^{**}P < 0.01$, compared to baseline.

lar function. Those actions appear to be related to the inhibition of the central nervous system (23, 25, 26). Our result shows that MAP was increased about 25% in pentobarbital-anesthetized mice treated with 1 mg/kg of cocaine. Based on the characteristics of the powerful sympathomimetic response of cocaine and our lab's unpublished data that cocaine increased MAP about 50%–100% in the ferret model, we concluded that an increase in blood pressure by cocaine in the mouse was masked by anesthetic agents. Also, Wilkerson (22) reported that a 1-mg/kg dose of cocaine increased mean blood pressure about 46 mmHg in conscious dogs, while only increasing blood pressure about 10 mmHg in pentobarbital anesthetized dogs.

Although researchers reported α-chloralose had a less depressant effect compared with other anesthetic agents (17, 23), our experiments and those of Faber (25) demonstrated that CU shows a similar depressant effect as pentobarbital on the cardiovascular system. In response to 1 mg/kg cocaine administration, MAP increased 19% in the CU mouse, whereas MAP in PEN mice increased by 35%. With a 3-mg/kg dose of cocaine, MAP was significantly increased in the PEN group ($P < 0.01$). However, in the CU group only MAP increased 7% (not significant). CU and PEN had a similar basal hemodynamic effect, but the cardiovascular response to cocaine was different. The PEN group seemed more sensitive than the CU group to cocaine's effects. Faber *et al.* reported that the combination of α-chloralose and urethane not only decreased MAP and HR, but also inhibited muscle contraction; however, the inhibition of PEN on muscle contraction was not marked at low dose (25, 27). Moreover, Bond *et al.* found that in the rat, anesthesia with pentobarbital was better able to compensate for acute blood loss and maintain blood pressure compared to α-chloralose-urethane (26). This may explain the different responses of CU and PEN to cocaine.

Data from the present study indicate that ketamine and xylazine were appropriate agents for the *in vivo* study of cardiovascular function in the mouse model. In the KX-anesthetized mice, the basal hemodynamic parameters, MAP and systolic LVP, were similar to conscious mouse data reported by other investigators using vessel cannulation (17–19). MAP was markedly higher than recorded in the CU and PEN groups ($P < 0.01$). However, HR in the KX group was significantly lower than both the PEN and CU groups. Reports in literature indicate that general anesthetics cause the HR to decrease from 40%–51% compared to conscious mice (17, 18) consistent with our observed 45% decrease. MAP was significantly increased by a 1-mg/kg dose of cocaine in KX-anesthetized mice. The time-response and dose-response curves were similar to CU and PEN.

In all three anesthetic groups, 5 mg/kg cocaine caused most mice to die within 1–3 min after administration. After bolus injection of 5 mg/kg cocaine, first the blood pressure significantly increased and then dropped below 20 mmHg. Cardiac arrhythmias were not observed during the whole period from beginning of cocaine injection to death. It appears that cardiac arrhythmia is not the primary cause of death in those animals. The cardiovascular collapse caused by a high dose of cocaine is possibly a result of direct depression of myocardial and vascular function due to cocaine's local anesthetic properties, as well as depression of the medullary vasomotor center (30).

In studying the effects of cocaine on the cardiovascular system, we observed the actions of acute doses of 1 mg/kg (i.v., bolus) on cardiac function in KX-anesthetized mice. From 1–3 min after administration of cocaine, LVEDP was elevated, and systolic LVP and +dP/dt fell significantly. The inhibitory effect of cocaine on ventricular function may result from sympathetically mediated vasoconstriction or from its direct negative chronotropic and inotropic actions. Although, cocaine can cause coronary spasm and reduce coronary blood flow (8, 9), studies (20, 31–34) reported that +dP/dt fell after cocaine administration whereas coronary blood flow was either unchanged or actually increased. Doses of 0–50 mg/kg cocaine were found to reduce left ventricular function without inducing coronary spasm and increasing systemic vascular resistance. Clinical studies demonstrated no evidence of thoracic aorta or coronary artery vasoconstriction immediately after i.v. cocaine; even subacute (15–60 min after administration) cocaine caused a reduction in coronary artery diameter (35). We considered that the negative inotropic effect of cocaine might be caused by direct depression of the left ventricle and not result from coronary vasoconstriction and ischemia. Moreover, cardiac conduction slowing by cocaine may contribute the negative inotropic effect (36, 37). As discussed above, 1-mg/kg cocaine significantly increased blood pressure after 3 min of cocaine administration; however, ventricular function was decreased. The time-response curves were not coincidental between blood pressure and LV function. These support the conclusion that the depressant effect of cocaine on ventricular function resulted from cocaine's direct negative inotropic effect on the heart and increase in systemic vascular resistance, independent of a reduction in coronary blood flow.

Compared to large animals, the mouse is significantly smaller in size and has a smaller blood volume. In general, loss of blood or tissue fluid during catheterization and experimental observation are unavoidable. Slight loss of body fluid such as evaporative loss in the mouse may cause a marked decrease of cardiovascular function. In our time-control experiment, blood pressure decreased after 90 min of catheterization ($P < 0.01$ compared to baseline). The results suggested that all cardiovascular pharmacological or physiological studies should be completed within 1 hr after catheterization in the *in vivo* mouse model.

In summary, our data demonstrated that general anesthetics significantly depress the cardiovascular response to cocaine in the mouse model. The sensitivity to cocaine in the mouse was less than that reported in the pig, dog, ferret, and rat. Our data suggest that a combination of ketamine-xylazine may be a suitable anesthetic agent for cardiovascular study in the mouse model. Also, the study observed that blood pressure declined spontaneously in the mouse model and that the rate of decline accelerated after 1 hr.

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