A comparison of the effects of ketamine, chloral hydrate and pentobarbital sodium anesthesia on isolated rat hearts and cardiomyocytes

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\textbf{Objectives} The study was intended to investigate which commonly used anesthetic in intact animals has the least effect on the function of isolated hearts and cardiomyocytes among the anesthetized animals.

\textbf{Methods} The hearts of male Sprague–Dawley rats were removed after they were anesthetized with ketamine, chloral hydrate or pentobarbital sodium, respectively, or were cervically dislocated. They were mounted on a Langendorff shelf. Heart rate (HR), left ventricular systolic pressure (LVSP), and maximal rate of increase of left ventricular pressure ($\frac{dp}{dt}$) were observed and recorded. Cell shorting amplitude and survival rate were detected in isolated cardiomyocytes.

\textbf{Results} The application of ketamine and pentobarbital sodium led to a significant decrease in HR, LVSP and $\frac{dp}{dt}$ in isolated hearts. Furthermore, pentobarbital sodium inhibited cell shorting amplitude and reduced the survival rate of isolated cardiomyocytes. Chloral hydrate did not significantly alter HR, LVSP, $\frac{dp}{dt}$, cell shorting amplitude and survival rate.

\textbf{Conclusion} The effects of anesthetics on cardiac parameters were considered when choosing an anesthesia administration. The results suggested that chloral hydrate as an anesthetic was appropriately applied for the studies of isolated hearts and cardiomyocytes.

\textbf{Keywords:} cardiomyocytes, chloral hydrate, isolated heart, ketamine, pentobarbital sodium

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\textbf{Introduction} With the progression of modern biotechnologies and the development of cardiovascular research, isolated rat hearts and cultured cardiomyocyte preparations are widely applied to the study of molecular and cellular mechanisms in many situations.\textsuperscript{1} In these studies, such reagents as ketamine, chloral hydrate and pentobarbital sodium are commonly used to anesthetize the animals. Previous studies found these anesthetics affected cardiac function in intact animals.\textsuperscript{2,–4} Pentobarbital sodium depressed myocardial function by reducing heart rate (HR), stroke index and cardiac index in male rats and mice.\textsuperscript{5,–8} Ketamine typically increases blood pressure, HR and cardiac output at the normal dose. It has been reported that ketamine induced depolarization of resting membrane potential in rat mesenteric artery myocytes, which may contribute to the rise in blood pressure.\textsuperscript{9} Chloral hydrate depressed the cardiovascular system in intact rats. As the depth of anesthesia augmented, cardiovascular depression increased which was reflected in the decrease in blood pressure, HR and arterial blood pH.\textsuperscript{10}

However, little was known about the effects of above-mentioned anesthetics on isolated rat hearts and cardiomyocytes. Until now, there have been no comprehensive comparisons among these three anesthetics (ketamine, chloral hydrate and pentobarbital sodium) in experimental cardiology.

Therefore, the present study was designed to compare the effects of ketamine, chloral hydrate and pentobarbital sodium on the functions of isolated hearts and cardiomyocytes, and the survival rate of cardiomyocytes was also determined. Hence, the aim of this study was to compare the effects of these agents on HR, left ventricular systolic pressure (LVSP) and maximal rate of increase of left ventricular pressure ($\frac{dp}{dt}$) of isolated hearts, and to compare the influence of these anesthetics on shorting amplitude and survival rate of cardiomyocytes. Also, and more importantly, we aimed to find a suitable anesthetic, which had the least interference with the functions of isolated hearts and cardiomyocytes for cardiovascular research.

\textbf{Materials and methods}

\textbf{Animals, anesthesia and preparation of isolated hearts} Approval of these experiments was obtained in advance from the Animal Ethics Committee of the Medical...
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College of Xuzhou. Male Sprague–Dawley rats (Clean grade, Xuzhou Medical College), weighing 180–200 g, were assigned to control group, ketamine group, chloral hydrate group and pentobarbital group. For the control group, rats were treated with heparin [5000 units/kg, intraperitoneally (i.p.)]. Ten minutes later, the rats were killed by cervical dislocation. For the remaining groups, all rats were treated with heparin (5000 units/kg, i.p.) and 10 min later, they were anesthetized with ketamine (100 mg/kg, i.p.) in the ketamine group, chloral hydrate (300 mg/kg, i.p.) in the chloral hydrate group and pentobarbital sodium (50 mg/kg, i.p.) in the pentobarbital group, respectively. About 20–30 min later, the adequate depth of anesthesia was achieved once the tail pinch and response to the corneal reflex disappeared. Then the hearts were quickly removed, mounted on a Langendorff set, and perfused with Krebs–Henseleit (KH) solution, which contained (mmol/l) 120 NaCl, 4.7 KCl, 1.2 KH$_2$PO$_4$, 1.2 MgSO$_4$, 25 NaHCO$_3$, 1.25 CaCl$_2$ and 11 glucose; the solution was adjusted to pH 7.4 by gassing with 95%O$_2$–5%CO$_2$. The hearts were perfused retrogradely at a flow rate of 6–10 ml/min. The temperature of the perfusion was maintained at 37°C. All hearts were stabilized for 10 min.

Assessment of cardiac function
Hearts were mounted on a Langendorff shelf. A balloon attached to a catheter was inserted into the left ventricle via the mitral valve. After stabilization for 10 min, HR, LVSP and $+dp/dt$ were monitored continuously by BL-420S (Chengdu, TME Technology Co. Ltd, Chengdu City, China).

Measurement of contraction in isolated ventricular myocytes
Cardiac myocytes were isolated as described before. The cardiomyocytes were added to an open chamber placed on the stage of an inverted microscope (Olympus Corp, Tokyo, Japan). Within 5 min, myocytes spontaneously attached to the bottom of the chamber, which was filled with KH solution and $10^{-7}$ mol/l isoprenaline. KH solution flowing at a speed of 2 ml/min was adjusted to pH 7.4 by equilibrating with 95%O$_2$–5%CO$_2$. The myocytes selected in the experiment were rod-shaped with clear sarcomeres. They were paced with an electric field stimulation (0.5 Hz). The images of myocyte contraction were transmitted to a computer. A video edge detector system in the computer measured the amplitude of myocellular shortening by spotting changes in the position of the edges of myocytes. The shortening amplitude was indexed as the percentage reduction of resting cell length following stimulation.

Identification of rod-shaped cells
Rod-shaped cells are the viable cells, and round-shaped cells are nonviable cells. The cells were counted under an inverted microscope. Five micrographs were taken randomly per sample, and all rod-shaped and round-shaped myocytes in these fields were measured. A total of 300–400 cells were counted for one condition of one preparation. The percentage of rod cells in total cells was calculated. The analysis of the cells was done under blind conditions.

Statistical analysis
All data are expressed as means ± SD and were analyzed by one-way or two-way analysis of variance (ANOVA) followed by Bonferroni post-hoc tests. A value of $P$ less than 0.05 was considered statistically significant.

Results
Hemodynamic effects of ketamine, chloral hydrate and pentobarbital sodium on isolated rat hearts
Compared with the control group, $+dp/dt$ and LVSP showed a marked reduction, and HR became slow in the ketamine group and the pentobarbital group ($P < 0.01$, both). But in the chloral hydrate group, $+dp/dt$, LVSP and HR were unchanged or declined slightly ($P > 0.05$; Fig. 1). These results suggested that ketamine and pentobarbital sodium weakened left ventricular systolic function and depressed activities of the heart, whereas chloral hydrate had the least hemodynamic effects on isolated rat heart among the three anesthetics mentioned.

Effects of ketamine, chloral hydrate and pentobarbital sodium on shortening and survival of isolated cardiomyocytes
To examine whether the three anesthetics used further influence cardiac function, shortening amplitude and survival rate of single cardiomyocytes were detected. Indeed, shortening amplitude and survival rate were significantly lower in the pentobarbital group than that in the control group ($P < 0.05$, Figs 2 and 3). These results suggested that pentobarbital sodium inhibited the contraction of single cardiomyocytes and accelerated the cell loss.

Discussion
In many experiments, ketamine, chloral hydrate and pentobarbital sodium are anesthetics most normally used to anesthetize the animals. Some anesthetics are proven to have interfering effects on the cardiovascular system, and they could lead to the misinterpretation of experimental outcomes. To gain reliable experimental data, the influence of anesthetics should be eliminated. In intact animals, the effects of some anesthetics on the cardiovascular system have been reported, but there have been few investigations into the effects of anesthetics on isolated hearts or cardiomyocytes.

In the present study, we compared the effects of ketamine, chloral hydrate and pentobarbital administered in intact animals on HR, LVSP and $+dp/dt$ of isolated rat
hearts, and on the contraction and the survival rate of cardiomyocytes. This initially studied the effects of normal anesthetics on isolated cardiomyocytes. The results indicated that ketamine, pentobarbital sodium and chloral hydrate depressed cardiac function in isolated rat hearts. The degrees of HR and \(+\frac{dp}{dt}\) depression were different: ketamine had the greatest effect, followed by pentobarbital sodium and chloral hydrate.

The experiment demonstrated that ketamine decreased HR from 320 to about 200 bpm in the first 15 min, and kept HR stable during the subsequent 15 min. In the ketamine group, \(+\frac{dp}{dt}\) was significantly lower than that in the remaining groups (\(P < 0.01\)), and LVSP diminished moderately. This indicated that ketamine produced a negative inotropic effect on isolated heart. But in intact animals or persons, it was found ketamine increased arterial pressure and HR.\(^{6, 17}\) It was reported that the

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**Fig. 1**

![Graphs showing effects of ketamine, chloral hydrate, and pentobarbital sodium on heart rate (a), LVSP (b), and contraction (c)](image)

Effects of ketamine, chloral hydrate, and pentobarbital sodium on heart rate (a), left ventricular systolic pressure (LVSP) (b), and contraction (c) of isolated cardiomyocytes. All data are expressed as means ± SD; \(n = 10\) hearts in each group. *\(P < 0.05\) vs. control group; **\(P < 0.01\) vs. ketamine group; ***\(P < 0.01\) vs. chloral hydrate group.

**Fig. 2**

![Bar graphs showing survival rate of isolated cardiomyocytes](image)

Effects of ketamine, chloral hydrate, and pentobarbital sodium on the survival of isolated cardiomyocytes. All data are expressed as means ± SD; \(n = 10\) hearts in each group. *\(P < 0.05\) vs. control group.

**Fig. 3**

![Bar graphs showing shortening of isolated cardiomyocytes](image)

Effects of ketamine, chloral hydrate, and pentobarbital sodium on the shortening of isolated cardiomyocytes. All data are expressed as means ± SD; \(n = 10\) hearts in each group. **\(P < 0.01\) vs. control group.
levels of plasma epinephrine and norepinephrine rose after administration of ketamine, which are concomitant with the increase in blood pressure and HR.\textsuperscript{18} So the increase in blood pressure and HR were secondary to the sympathetic stimulation caused by ketamine. We suspected that the decline of HR and $+\text{dp/dt}$ in isolated hearts was the direct effect of ketamine. It was possible that the direct inhibition of ketamine on hearts was covered up by its stimulation of the sympathetic system in intact animals. So it can be concluded that ketamine produced direct suppression of isolated hearts. Therefore, this agent is not appropriate for hemodynamic research.

In the present study, pentobarbital sodium administered in vivo depressed cardiac activities, as indicated by a significant decrease in LVSP, $+\text{dp/dt}$ and HR in isolated hearts. These findings confirmed in part the observations of Snyder et al.,\textsuperscript{15} who stated that a high dose of pentobarbital produced an inhibitory effect on isolated heart preparations. Clearly, the depressed myocardial function caused by pentobarbital sodium should be reason enough to exclude this anesthetic from use in such studies. Recently, a study by Zorniak et al.\textsuperscript{19} demonstrated that pentobarbital is the most suitable anesthetic for cardiovascular studies because of its slight disturbance on hemodynamic parameters (including HR, mean arterial blood pressure) in the reperfusion-induced arrhythmia model in rats in vivo. The discrepancy between the present results and the previous study may be attributed to different models used in the experiments or distinguishing effects of pentobarbital on isolated heart and in-situ heart.

Regarding the mechanism of action, it should be remembered that the negative inotropic effect of pentobarbital appears to be related to myocardial calcium regulation. Pentobarbital was proven to disrupt Ca\textsuperscript{2+} movements between the cytosol and the extracellular fluid.\textsuperscript{20} The decreasing HR probably results from inhibition of potassium channels by pentobarbital sodium.\textsuperscript{21}

Our study delivered the novel findings that pentobarbital sodium weakened the response of cardiomycocytes to isoprenaline. Furthermore, it was found that pentobarbital caused a significant loss of cardiomycocytes. Only about 30% of cardiomycocytes were viable in the pentobarbital group, whereas approximately 80% were viable in the control group ($P < 0.01$). This is the first study to demonstrate the inhibitory effect of pentobarbital on single cardiomycocytes. The underlying mechanisms remain unclear and deserve further investigation.

In conclusion, our study delivers new information about anesthetics widely used in cardiovascular studies, which should be taken into consideration by researchers. Among tested agents, chloral hydrate anesthetics offered stable hemodynamic conditions and had no adverse effect on contraction and survival of cardiomycocytes, which were distinctly different from those exhibited with ketamine and pentobarbital sodium. Considering all our results together, we suggest that chloral hydrate appears to be the most appropriate agent for heart studies.

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**References**
