Time-Dependent Change in Baroreflex Control Capacity of Arterial Pressure by Pentobarbital Anesthesia in Rabbits

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Abstract: The present study is designed to investigate the time-dependent effect of pentobarbital anesthesia on the baroreflex arterial pressure (AP) control system in rabbits. The overall AP control capacity of the baroreflex system was assessed with mean arterial pressure (MAP) responses to the rapid mild hemorrhage (2 ml/kg body weight) and an overall open-loop gain (G) of the system. The G value was determined by means of the following formula: $G = \Delta AP_i / \Delta AP_s - 1$, where ΔAP_i is an immediate MAP fall and ΔAP_s a steady-state fall after the rapid hemorrhage. Prior to the experiment, two catheters for AP measurement and hemorrhage were chronically in-dwelt in the aortic arch via the left subclavian and left common carotid arteries, respectively. Control mean arterial pressure averaged for 30 sec before the rapid hemorrhage (CAMP), ΔAP_i and ΔAP_s significantly increased and reached the maximal value at 14 min (CAMP: $p < 0.01$) and 28 min (ΔAP_i : $p < 0.01$ and ΔAP_s : $p < 0.01$) after the intravenous injection of sodium pentobarbital in a 25.0 mg/kg dose, respectively. These values gradually decreased in the course of time and tended to recover to near the preanesthetic level at 77–98 min after the anesthesia. The G value significantly decreased from 7.3 in the conscious state to 1.5 at 28 min after the anesthesia ($p < 0.001$), gradually increased with lapse of time and recovered to near the preanesthetic level at 77–98 min after the anesthesia. No significant difference in G was observed between in the conscious and anesthetized states beyond 70 min after the anesthesia ($p > 0.05$). These findings suggest that pentobarbital sodium exerts a time-dependent inhibitory effect on the baroreflex system but does not significantly affect the overall AP control capacity of the baroreflex system itself at least 70 min after the intravenous administration at a dose of 25.0 mg/kg.

Key words: baroreflex system, open-loop gain, pentobarbital sodium, rabbit

Introduction

Pentobarbital sodium has often been used for cardiovascular study in veterinary and pre clinical medicine. It is known that pentobarbital anesthesia can affect cardiovascular function, whereas it decreased fluctuation in cardiovascular parameters i.e., arterial pressure (AP) and heart rate (HR), and so forth, establishing stable experimental conditions.

Pentobarbital anesthesia has been indicated to play a major part in the autonomic nervous system [3, 5, 16, 19, 21, 22], which is responsible for modifying the sensitivity of baroreflex AP or HR control [1, 2, 8, 16]. Zimpfer, *et al.* [24] reported that pentobarbital sodium reduced the capacity to restore hypotension due to hemorrhage. On the other hand, Hosomi and Sagawa [8] and we [13] demonstrated that pentobarbital sodium did not affect the baroreflex AP control capacity after hemorrhage. The reason for the discrepancy in the effect of pentobarbital anesthesia on the control capacity of the baroreflex AP control system, although associated with many factors, remains to be clarified.

The effect of anesthetics in general gradually diminishes after administration, accompanied with a decrease in the concentration in plasma, though it depends on dosage, species, age and other conditions. The AP level tends to return to the preanesthetic level with time after the anesthesia. The time from the administration of anesthetic to starting the experiment has not been clearly reported in most studies. This would be one of the important factors in the disparity among investigations in the effect of pentobarbital anesthesia on the circulatory system.

In the present study, we investigate the time-dependent effect of pentobarbital sodium on the baroreflex AP control capacity and compare it with that under conscious conditions in rabbits. The control capacity of the baroreflex system was assessed by the mean arterial pressure (MAP) responses to rapid mild hemorrhage and the overall open-loop gain (G) of the system.

Materials and Methods

Animals: Fourteen normal Japanese white conventional rabbits (body weight; 3.1 ± 0.4 kg and age; 18.5 ± 3.8 months old, mean $\pm$ SD, Fujii Animals, Co., Hyogo, Japan) were used in the present study. They

were bred in an individual wire cage with a commercial pellet diet (ORC-4, Oriental Yeast, Co., Ltd., Japan) at 100 g/day in a room with the temperature at 22–25°C and relative humidity at 50–60%.

Experimental protocol: A schematic arrangement of a laboratory animal and setup are shown in Fig. 1. The rabbits were anesthetized with an intravenous injection of pentobarbital sodium at a dose of 30.0 mg/kg. Two large bore catheters (1.2 mm, I.D.) for AP measurement and rapid mild hemorrhage were chronically placed into the aortic arch through the left subclavian and the left common carotid arteries, respectively. The other ends of both catheters were subcutaneously tunneled to the back and connected to stop cocks. The catheters were filled with heparin (Novo heparin, Kodama, Co., Ltd., Japan) every day at a dose of 500 U/kg to prevent blood coagulation. Several days after the catheterization, we confirmed that the rabbits were active and in a good state of health. They were transferred from the previous cage to the small experiment cage. Two hours was allowed to acclimate the animal to the experiment environment. MAP in the aortic arch was measured with a catheter, transducer (MPU-0.5A, Nihon Kohden Kogyo, Co., Ltd., Japan) and preamplifier (AP-6200, Nihon Kohden Kogyo Co., Ltd., Japan) through a low-pass filter with a time constant of 2 sec. A small amount of blood (2 ml/kg body weight) was withdrawn from the aortic arch into a syringe with a catheter within 1–2 sec as quickly as possible under

Fig. 1. Schematic arrangement of laboratory animal and experimental set-up.

conscious conditions. MAP response to the rapid mild hemorrhage was recorded with a pen-recorder and simultaneously fed into a computer (JEC-7E, JEOL Ltd., Japan) through an analogue-to-digital converter at intervals of 100 msec for 30 sec before and 120 sec after the hemorrhage. The shed blood was slowly re-infused via the hemorrhage catheter 3 min after the rapid hemorrhage. This hemorrhage-infusion procedure was repeated 8 times for each animal. After the hemorrhage experiment in the conscious state, the rabbits were anesthetized again with an intravenous administration of pentobarbital sodium at a dose of 25.0 mg/kg to evaluate the effect of pentobarbital sodium on the baroreflex AP control system. Rapid mild hemorrhage was started 7 min after the anesthesia at intervals of 7 min for 98 min.

Data analysis: The computer-stored strings of MAP responses under conscious and anesthetized (77–98 min after the onset of anesthesia) conditions were averaged 4 and 8 times to improve the signal-to-noise ratio due to changes in posture, and other factors, respectively.

Fig. 2. Example of MAP response to rapid mild hemorrhage (A) and schematic illustration of the feedback AP control of the baroreflex system (B). The open-loop gain (G) of the system was determined as $\Delta AP_i / \Delta AP_s - 1$, where ΔAP_i was an immediate MAP fall after rapid hemorrhage and ΔAP_s a steady-state MAP fall 1–2 min after hemorrhage.

Control mean arterial pressure (CMAP) was averaged in MAP for 30 sec before the rapid hemorrhage. Fig. 2 shows a typical MAP response to the rapid mild hemorrhage (A) and determination of G of the overall baroreflex AP control system (B). ΔAP_i was taken as the peak of instantaneous MAP fall immediately after the rapid hemorrhage and ΔAP_s was measured as a steady-state fall observed 1–2 min after the hemorrhage. G was assessed on the basis of the linear control theory [6, 7, 9, 15] by the following formula: $G = \Delta AP_i / \Delta AP_s - 1$. The data were analyzed by Tukey's multi-comparison test for preanesthetic and postanesthetic conditions at each time after confirming significant difference by analysis of variance.

Results

Figure 3 shows example recordings of MAP response to the rapid mild hemorrhage before and after pento-

Fig. 3. Typical MAP responses to rapid mild hemorrhage in the conscious and anesthetized states. Pentobarbital sodium was intravenously administered at a dose of 25.0 mg/kg. The responses averaged 8 and 4 times in the conscious and anesthetized (77–98 min after the anesthesia) states to improve the signal to noise ratio, respectively.

barbital anesthesia. In the response under conscious conditions, MAP transiently fell immediately after the rapid hemorrhage and thereafter recovered to near the prehemorrhage level with a slight steady-state fall. Immediate MAP fall (ΔAP_I) considerably increased from 14 to 63 min after the anesthesia. The steady-state MAP fall (ΔAP_S) increased after the anesthesia, reached its maximum at 28 min after the anesthesia and thereafter gradually decreased.

Figure 4 shows changes in CMAP plotted as a function of time. CMAP was suddenly increased from 90.8 on average in the conscious state to 102.4 mmHg at 14 min ($p < 0.01$), gradually decreased with time, and almost returned near the preanesthetic level at 77–98 min after the anesthesia. No significant difference was observed at 77–98 min after the anesthesia ($p > 0.05$). Time-dependent changes in ΔAP_I and ΔAP_S induced by pentobarbital anesthesia are shown in Figs. 5 and 6, respectively. The values for ΔAP_I and ΔAP_S were 9.2 and 1.1 mmHg under conscious conditions, respectively. ΔAP_I significantly increased immediately after the anesthesia, reached the maximal value of 18.9 mmHg at 28 min ($p < 0.01$), remained high and gradually decreased

from 56 min after the anesthesia. There was no significant difference in ΔAP_I at 77–98 min after the anesthesia, though the value was relatively high in comparison with that under preanesthetic conditions. ΔAP_S rose to 8.3 mmHg at 28 min after the anesthesia and thereafter gradually decreased to around the preanesthetic level. No significant difference in ΔAP_S between in the conscious and anesthetized states was observed from 63 min after the anesthesia.

Figure 7 depicts a change in G with time. G significantly decreased from 7.3 in the conscious state to 1.5 on average at 28 min after the anesthesia ($p < 0.001$) and gradually recovered with time. G returned to near the preanesthetic level at 77–98 min after the anesthesia. There was no significant difference between G in the conscious state and that in the anesthetized state from 70 min after the anesthesia ($p > 0.05$).

Discussion

There is a volume of research literature on the effects of pentobarbital anesthesia on cardiovascular function. Although most investigators have observed

Fig. 4. Time-dependent change in CMAP after intravenous administration of pentobarbital sodium at a dose of 25.0 mg/kg. CMAP was calculated as an average in MAP for 30 sec before rapid mild hemorrhage. CMAP at 0 and 77– min were determined from the MAP response to rapid hemorrhage averaged 8 and 4 times under conscious and anesthetized (from 77 to 98 min after the anesthesia) conditions, respectively. Significant difference between the preanesthetic and anesthetized states at each time was examined by Tukey's multi-comparison test.

Fig. 5. Time-dependent change in ΔAP_1 after intravenous administration of pentobarbital sodium at a dose of 25.0 mg/kg. ΔAP_1 at 0 and 77–min were determined from averaged MAP response to rapid hemorrhage 8 and 4 times under conscious and anesthetized (from 77 to 98 min after the anesthesia) conditions, respectively. Significant difference was tested as shown in Fig. 4.

Fig. 6. Time-dependent change in ΔAP_s after intravenous administration of pentobarbital sodium at a dose of 25.0 mg/kg. ΔAP_s at 0 and 77–min were determined from averaged MAP response to rapid hemorrhage 8 and 4 times under conscious and anesthetized (from 77 to 98 min after the anesthesia) conditions, respectively. Significant difference was tested as shown in Fig. 4.

an increase in AP in acute animal studies, other investigators [4, 8, 14, 24] have reported that the AP level was not significantly different from the control level. Fray *et al.* [4] and Zimpfer *et al.* [24] suggested that

this difference would result from that between the animals used i.e., trained or untrained, and that excitement and surgical trauma in introducing an untrained animal to general anesthesia would also be responsible for a

Fig. 7. Time-dependent change in G after intravenous administration of pentobarbital sodium at a dose of 25.0 mg/kg. G at 0 and 77– min were determined from averaged MAP response to rapid hemorrhage 8 and 4 times under conscious and anesthetized (from 77 to 98 min after the anesthesia) conditions, respectively. Significant difference was tested as shown in Fig. 4.

rise in the AP level. The effects of pentobarbital sodium on the cardiovascular regulatory system have been studied to investigate the mechanism of circulatory action of the anesthetics. Pentobarbital sodium has been shown to have the suppressing effects on the sympathetic [5, 16, 21, 22] or parasympathetic nervous system [5, 19], which involved the reduction in baroreflex function.

The effect of pentobarbital sodium on the baroreflex system has often been evaluated with AP or HR responses to cardiovascular stimulus, e.g., hemorrhage. Vatner and Braunwald [23] showed a difference in AP control function after hemorrhage in the conscious and anesthetized states. Cox and Bagshaw [2] demonstrated that pentobarbital sodium modified cardiovascular responses to carotid hypotension. On the other hand, Hosomi and Sagawa [8] reported no effect of pentobarbital anesthesia on the baroreflex control capacity of AP after the hemorrhage of 10% of blood volume within 20–30 sec in dogs. Zimpfer *et al.* [24] carried out a hemorrhage experiment with the same protocol as that of Hosomi and Sagawa [8] to reconfirm the effect of pentobarbital anesthesia, but it failed to be consistent with their conclusions. This discrepancy has been controversial.

Thus, the effect of pentobarbital anesthesia on the baroreflex system is at variance among investigators. The disparity in the cardiovascular effects of pentobarbital sodium would arise from the difference in the assessment of baroreflex function or that in the process of recovery from the anesthesia, in addition to the differences in dosage, species, age and other experimental conditions. The amount of hemorrhage and analysis of cardiovascular response to the hemorrhage seem to be important factors in precisely assessing the cardiovascular control capacity of the baroreflex system.

The AP control capacity of the baroreflex system has often been evaluated with a G value determined from AP responses to hemorrhage by the application of the control theory to the system [15]. Most investigators have employed massive hemorrhage to determine G in dogs. Massive hemorrhage causes a large decrease in blood volume, resulting in abnormal blood distribution to the organs and a decrease in systemic blood pressure. In some cases, the animals could show shock-like manifestations. The hypovolemia could affect humoral or hormonal cardiovascular regulatory mechanisms and cause hypoxia, acidosis, and so forth. Hosomi *et al.* [10] previously assessed the time-dependent changes in the AP control capacity of the baroreflex system after

massive hemorrhage (5 ml/kg, body weight) with the open-loop gain determined by rapid mild hemorrhage. The control capacity of the baroreflex AP control system progressively decreased with time after massive hemorrhage and tended to recover somewhat by 110 min after hemorrhage. Therefore, the evaluation of the baroreflex AP control capacity under the hypovolemic conditions due to massive hemorrhage, including estimation of time-dependent effects of anesthetics, is not considered to show the precise control capacity of the system because massive hemorrhage must be a parametric forcing which modifies the normal characteristics of the baroreflex AP control system [6–9, 16–18].

On the other hand, rapid mild hemorrhage is the withdrawal of a small amount of blood to allow us to do a precise physiological evaluation of baroreflex function [6, 7, 9–11, 13]. The MAP fall immediately after rapid mild hemorrhage (ΔAP_I) is a transient fall in AP due to a small decrease in blood volume in the aorta. ΔAP_I is observed in animals whose AP feedback baroreflex control system has been completely eliminated [9]. The baroreflex system operates at least about 2 sec after the application of external disturbances, such as hemorrhage, to the system [20]. Immediately after the rapid mild hemorrhage, the baroreflex system does not function [20]. These facts suggest that ΔAP_I is in major part determined by cardiovascular mechanical properties, such as distensibility of the aortic wall, capacity of the aorta, rate of filling the aorta with blood, rate of draining blood from the aorta to the peripheral vessels, and so forth. The baroreflex system functions most powerfully 1–2 min after the hemorrhage [9]. The MAP fall (ΔAP_S) is a steady-state error in the system which is observed in each kind of hemorrhage experiment irrespective of the hemorrhage volume. ΔAP_I and ΔAP_S must be a physiological test input and an output in the baroreflex system, respectively. Therefore, the baroreflex system could be considered to be a proportional negative-feedback control system. The input-output relationship determined by rapid mild hemorrhage can estimate the normal characteristics of the baroreflex system. In previous reports, the values for overall open-loop gain of the baroreflex system estimated by method of the rapid mild hemorrhage were 7.4 and 7.8 in anesthetized rabbits [11] and dogs [10] with pentobarbital sodium in intravenous doses of 27.5 and 35.0 mg/kg, respectively. These suggest that there

is no remarkable difference between the two species in the overall AP control capacity of the baroreflex system in this dose range.

We previously compared the values for CMAP, ΔAP_I , ΔAP_S and G in the conscious state with those determined 60–90 min after the anesthesia in normal and Watanabe heritable hyperlipidemic (WHHL) rabbits, since the MAP responses were unstable for about 60 min after the onset of the anesthesia [13]. The G value resulted in showing no significant difference between the conscious and anesthetized conditions in either rabbit group. This suggests that the effect of pentobarbital anesthesia on the baroreflex system is time-dependent. In the present study, there were no significant differences in these values between the conscious and anesthetized states 77 min after the onset of the pentobarbital anesthesia. This means that the overall AP control capacity of the baroreflex system has almost recovered from the effect of pentobarbital anesthesia 77 min after the anesthesia at a dose of 25.0 mg/kg. The time-dependent change in CMAP after the anesthesia did not parallel those of ΔAP_S and G though there were no statistical significant differences between their preanesthetic level and that 77 min after the anesthesia. CMAP and ΔAP_I rose about 1.1 and 2.1 fold of the preanesthetic value at maximum, respectively, whereas ΔAP_S and G showed a larger increase and decrease about 7.4 and 0.2 fold of the preanesthetic value at maximum, respectively, in comparison to changes in CMAP and ΔAP_I . Although the site of action of pentobarbital sodium was not investigated in the present study, we can suppose that this anesthetic, restricted to circulatory function, would affect both the peripheral mechanical function of the heart and blood vessels and the central and peripheral nervous systems from the baroreceptor to the sympathetic and parasympathetic nerves because of changes in both the input and output values to the system caused by the anesthesia. The complicated central and peripheral actions of pentobarbital sodium would cause differences in maximal changes in ΔAP_I , ΔAP_S and G in response to rapid mild hemorrhage, which would in part be responsible for the disparity in the recovery rates of these variables from the effects of anesthesia.

We could compromise one of the discrepancies in the effect of pentobarbital anesthesia on the baroreflex AP control system in consideration of the time-depend-

ent change in the overall AP control capacity of the baroreflex system. The differences in the cardiovascular effects of pentobarbital sodium due to those in species, dosage and method of administration, e.g. intravenous, intraperitoneal or subcutaneous, should be elucidated in future. In evaluating cardiovascular function under anesthetized conditions, consideration of the time-dependent effect of anesthetics, as well as differences in species, dosage, animal conditions, sensitivity to anesthetics, and so forth could provide some useful information on the cause of variation in the measured cardiovascular parameters. Moreover, it is also important to reconfirm whether cardiovascular stimulus and the method used in analyzing the cardiovascular response to the stimulus precisely represent physiological regulatory function of the cardiovascular system or not.

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