Impaired Baroreflex Control of Arterial Pressure in WHHL Rabbits

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ABSTRACT. The present study was designed to investigate baroreflex control capacity of arterial pressure (AP) in the conscious Watanabe heritable hyperlipidemic (WHHL) rabbit. The control capacity of the baroreflex system was assessed with overall open-loop gain (G). Seven WHHL and 14 normal Japanese white rabbits were chronically implanted two catheters in the aortic arch through the left subclavian and common carotid arteries. A small amount of blood (2 ml/kg, body weight) was rapidly extracted into a syringe via the left common carotid artery in the conscious state. Mean arterial pressure (MAP) was monitored with a catheter-transducer system through the left subclavian artery. The MAP responses to the rapid hemorrhage were averaged 8 times by a computer. G was calculated as $G = \Delta AP_i / \Delta AP_s - 1$, where ΔAP_i was an immediate MAP fall after the hemorrhage and ΔAP_s was a steady-state error 1–2 min after the hemorrhage. The values of G in the conscious normal and WHHL rabbits were 7.35 ± 0.24 and 1.91 ± 0.29 (mean $\pm$ SE, $p < 0.01$), respectively. To investigate effects of pentobarbital anesthesia on baroreflex system, the hemorrhage experiment was repeated several times under pentobarbital anesthesia (20 mg/kg, i.v.). The values of G in the anesthetized normal and WHHL rabbits were 6.69 ± 0.23 and 1.68 ± 0.34 (mean $\pm$ SE, $p < 0.01$), respectively. G in the normal and WHHL rabbits did not show any significant change in the presence and absence of pentobarbital anesthesia ($p > 0.05$). It is concluded that the reduced baroreflex control capacity of AP was partly caused by decreased sensitivity of the baroreceptors due to spread of sclerotic lesions and that pentobarbital anesthesia did not affect overall AP control capacity of the baroreflex system.—**KEY WORDS:** arterial pressure, atherosclerosis, baroreflex, pentobarbital anesthesia, WHHL rabbit.

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In the previous paper [17], Watanabe heritable hyperlipidemic (WHHL) rabbit showed significant elevation of arterial pressure (AP) and significant increase in lability of AP under conscious conditions, which reflected pathological findings of the aorta. These suggest that AP control capacity of the baroreflex system should decline with development of atherosclerosis.

We [15] previously estimated open-loop gain (G) of the baroreflex system in the anesthetized WHHL rabbit. G was significantly smaller in the WHHL rabbit than in the normal rabbit. Pentobarbital anesthesia, however, may modulate cardiovascular control including arterial baroreceptor reflex after hemorrhage [6, 24, 27]. To the contrary, Hosomi and Sagawa [12] demonstrated that pentobarbital anesthesia exerted no effect on responsiveness of the baroreflex system. Thus, the effect of pentobarbital anesthesia is still controversial.

The present study was designed to quantify overall control capacity of the baroreflex system in the WHHL and normal rabbits in the conscious state. The control capacity was assessed with G determined from AP response to quick mild hemorrhage. To investigate effects of pentobarbital anes-

thesia on baroreflex function, G in the WHHL and normal rabbits in the conscious state were compared with those in the anesthetized state.

MATERIALS AND METHODS

Animals: Seven WHHL rabbits (age: 12 to 29 months, weight 3.08 ± 0.22 kg (mean $\pm$ SE)) and 14 normal Japanese white rabbits (age: 12 to 30 months, weight 3.43 ± 0.09 kg (mean $\pm$ SE)) were used in the present study. They were individually housed in stainless wire cage in the Experimental Animal Laboratory at temperature of 20–24°C and humidity of 50–60%, and freely accessed to a commercial rabbit food (ORC-4, Oriental Yeast, Co., Ltd.) at 100 g/day/animal.

Experimental procedure: The rabbits were anesthetized with pentobarbital sodium (Nembutal, Abbott Laboratories) in an intravenous dose of 25 mg/kg. Two catheters for AP measurement and hemorrhage were introduced into the aortic arch via the left subclavian and left common carotid arteries, respectively. The other end of each catheter was subcutaneously connected to a stopcock fixed on the rabbit's back. Before the hemorrhage experiment

began, heparin (Novo heparin, Kodama Co., Ltd.) was injected into the catheters every day in a dose of 500 U/kg. A few days were allowed to recover from the surgery. At a time of hemorrhage experiment, the rabbits were active and in a good state of appetite. They were housed in a small experimental cage and acclimatized to the experimental environment. Schematic arrangement of the rabbit and experimental apparatus was illustrated in Fig. 1. A small amount of blood (2 ml/kg, body weight) was rapidly withdrawn from the aorta to a syringe within 1 sec. AP at the aortic arch was monitored with a catheter-transducer system (MPU-0.5A, Nihon Kohden Kogyo). Mean arterial pressure (MAP) was derived from AP through a low-pass filter with a time constant of 2 sec. The MAP responses to the quick hemorrhage were recorded on a strip-chart and simultaneously sampled by a computer (JEC-7E, JEOL Ltd.) from 30 sec before to 2 min after the hemorrhage through an analogue-to-digital converter every 1 sec. The shed blood was reinfused into the aorta through the hemorrhage catheter. This experimental procedure was repeated 8 times on the identical animal in conscious conditions. Then, the rabbits were anesthetized again by the intravenous administration of pentobarbital sodium in a dose of 20 mg/kg. After stable AP had been established, the same experimental procedure was repeated several times.

Data analysis: The data strings of MAP response to the hemorrhage in each conscious rabbit were pooled in a computer and averaged 8 times. We measured control mean arterial pressure just before the hemorrhage (CMAP). MAP fell immediately after the hemorrhage (ΔAP_I). It was restored close to the control level and reached a new stable state 1–2 min after the hemorrhage (ΔAP_S) [13]. G of the negative feedback AP control system was estimated by the following formula: $G = \Delta AP_I / \Delta AP_S - 1$. Stu-

dent t -test was used for statistical analysis in the present study.

RESULTS

Figures 2 and 3 showed examples of MAP response to the quick mild hemorrhage in the conscious normal and WHHL rabbits, respectively. Since irregular AP fluctuation due to mainly postural change was observed in each trace, these MAP responses were averaged within individuals to improve signal-to-noise ratio. The results were shown in Fig. 4. In both normal (A) and WHHL (B) rabbits, AP fluctuation was reduced in comparison with the original records, which enabled us to measure CMAP, ΔAP_I and ΔAP_S precisely.

After the repetition of the hemorrhage experiment in conscious conditions, the rabbits were anesthetized again with pentobarbital sodium (20

Fig. 1. Schematic representation of experimental set-up.

Fig. 2. Original mean arterial pressure responses to the quick mild hemorrhage in the conscious normal rabbit.

Fig. 3. Original mean arterial pressure responses to the quick mild hemorrhage in the conscious WHHL rabbit.

Fig. 4. Averaged mean arterial pressure responses to the quick mild hemorrhage in the normal (A) and WHHL (B) rabbits in the conscious state. The responses in Figs. 3 and 4 were averaged 8 times, respectively.

mg/kg, i.v.) to estimate effects of pentobarbital anesthesia on the baroreflex system. CMAP was significantly increased by pentobarbital anesthesia in the normal rabbit, whereas it was not significantly elevated in the WHHL rabbits (Table 1). CMAP was significantly higher in the WHHL rabbit than in the normal rabbit in both conscious and anesthetized conditions (Table 1). ΔAP_I (Table 2) and ΔAP_S (Table 3) were significantly increased by pentobarbital anesthesia in both rabbits. ΔAP_I (Table 2) and ΔAP_S (Table 3) were significantly larger in the WHHL rabbit than in the normal rabbit in both conscious and anesthetized conditions. However, G value was not changed by pentobarbital anesthesia in both rabbits, whereas this value was

Table 1. CMAP values in the normal and WHHL rabbits in the conscious and anesthetized states

Rabbit	Normal	WHHL	p value
Conscious	90.79 $\pm$ 1.51	103.34 $\pm$ 3.13	p<0.01
Anesthetized	95.27 $\pm$ 1.39	105.61 $\pm$ 2.87	p<0.02
p value	p<0.05	p>0.05	

Each value represents mean $\pm$ SE (mmHg).

Table 2. ΔAP_I values in the normal and WHHL rabbits in the conscious and anesthetized states

Rabbit	Normal	WHHL	p value
Conscious	9.24 $\pm$ 0.57	17.90 $\pm$ 2.16	p<0.01
Anesthetized	15.18 $\pm$ 1.64	29.78 $\pm$ 1.99	p<0.01
p value	p<0.01	p<0.02	

Each value represents mean $\pm$ SE (mmHg).

Table 3. ΔAP_S values in the normal and WHHL rabbits in the conscious and anesthetized states

Rabbit	Normal	WHHL	p value
Conscious	1.09 $\pm$ 0.11	6.74 $\pm$ 1.14	p<0.01
Anesthetized	2.13 $\pm$ 0.18	12.05 $\pm$ 1.49	p<0.01
p value	p<0.01	p<0.01	

Each value represents mean $\pm$ SE (mmHg).

Table 4. G values in the normal and WHHL rabbits in the conscious and anesthetized states

Rabbit	Normal	WHHL	p value
Conscious	7.35 $\pm$ 0.24	1.91 $\pm$ 0.29	p<0.01
Anesthetized	6.69 $\pm$ 0.23	1.68 $\pm$ 0.34	p<0.01
p value	p>0.05	p>0.05	

Each value represents mean $\pm$ SE.

significantly smaller in the WHHL rabbit than in the normal rabbit in both conscious and anesthetized conditions (Table 4).

DISCUSSION

MAP response to the quick mild hemorrhage consists of two major components. ΔAP_I is an immediate MAP fall seen within 1–2 sec after the end of the hemorrhage. In this step, the nervous AP control system does not function [23]. Though baroreflex mediated changes in heart rate could influence the magnitude of initial fall in MAP, ΔAP_I is also observed in the animal denervated all of the baroreceptor nerves [11, 16]. Its size, therefore, depends on compliance of the arterial wall.

The WHHL rabbit genetically develops atherosclerosis which occurs in the ascending aorta by the age of 5 months and is spread over the arterial tree with aging [18, 25, 26]. Therefore, regression of atherosclerotic lesions can not be expected in the WHHL rabbit. Atherosclerosis alters vasoelastic characteristics of the arterial wall [5]. Hasegawa and Watanabe [8] demonstrated progressive reduction in distensibility of the atherosclerotic vessels in the WHHL rabbit aged 8 to 30 months. The present results show a significant increase in ΔAP_I in the conscious and anesthetized WHHL rabbits in comparison with those in the conscious and anesthetized normal rabbits. This would be caused by reduced compliance of the arteries due to growth in the size of plaques and spread of the atherosclerotic lesions.

Pentobarbital sodium, which has been often employed for animal studies on hemodynamics, produces a variety of effects on the cardiovascular system. In the present study, pentobarbital sodium induced a significant increase in ΔAP_I in the normal and WHHL rabbits. This suggests that pentobarbital sodium would decrease the compliance of the arteries.

Because baroreceptors respond to mechanical stretch of the vascular wall at which they locate, their responsiveness is directly affected by vasoelastic properties [1–4]. Therefore, atherosclerosis at the receptor site will modify the baroreflex function. Angell-James [2, 3] indicated that the carotid sinus and aortic arch baroreceptor activities were diminished in the rabbit with dietary induced or vitamin D sclerosis. Morita *et al.* [22] reported that both sino-aortic and cardiopulmonary baroreceptor reflexes mediated changes in renal nerve activity

were impaired in the WHHL rabbit. In the present study, the values of G were significantly lower in the WHHL rabbit than in the normal rabbit in the presence and absence of pentobarbital sodium. We could conclude that responsiveness of the carotid sinus and aortic arch baroreceptors were diminished by a decrease in distensibility of the arterial wall due to the spread of atherosclerosis to the baroreceptor regions.

Elevation of AP level by pentobarbital anesthesia is often observed in acute preparations in our usual laboratory work. To the contrary, there have often been reported several conflicting results that pentobarbital sodium did not significantly change preanesthetic AP level [7, 12, 19, 27]. Fray *et al.* [7] and Zimpfer *et al.* [27] suggested that this difference should be resulted from the use of the animal; untrained or trained. They reported that AP level was elevated by pentobarbital anesthesia in the untrained dog. In the present study, we used untrained rabbits and observed significant change in CMAP by pentobarbital anesthesia in the normal rabbit. This is in agreement with the suggestions by Fray *et al.* [7] and Zimpfer *et al.* [27]. However, CMAP was not significantly changed by pentobarbital anesthesia in the WHHL rabbit. The effects of pentobarbital on AP may be different between normal and pathological conditions.

Most investigators have been reported that pentobarbital anesthesia modified baroreflex control of AP after hemorrhage [6, 24, 27]. On the other hand, Hosomi and Sagawa [12] withdrew approximately 10% of blood volume within 20–30 sec in the dog under conscious and anesthetized conditions, indicating no effect of pentobarbital anesthesia on AP response to the hemorrhage. Zimpfer *et al.* [27] simulated the same protocol as that of Hosomi and Sagawa [12] but failed to show no difference in AP responses between in the presence and absence of pentobarbital anesthesia. The effects of pentobarbital anesthesia on baroreflex function have been in dispute. The present results show no significant change in G in the normal and WHHL rabbits by pentobarbital anesthesia. This is inconsistent with the results of Zimpfer *et al.* [27]. The difference seems to be due to that of experimental method. A large amount of hemorrhage may cause several pathophysiological responses. It must be a parametric forcing and disturb normal control capacity of the baroreflex system [9, 10, 14, 21], while a quick mild hemorrhage is not a parametric forcing but just a

test input [9]. On the other hand, pentobarbital anesthesia is also a parametric forcing [20]. If we simultaneously impose two parametric forcing on the biological systems, they may interact and cause unpredictable pathophysiological responses.

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