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# Effects of Acute Arterial Bleeding on Renal Sympathetic Nerve Activity in Young and Old Anesthetized Rats

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## ABSTRACT

We compared the change in renal sympathetic nerve activity (RNA) during hemorrhage in young and old rats. Young (10 weeks) and old (80 weeks) male Wistar rats were anesthetized, and RNA together with arterial pressure (AP) and heart rate (HR) were measured under spontaneous respiration. Acute arterial bleeding, 1ml/100g body weight, was carried out to induce hypotension and hypovolemia. With bleeding, mean AP (MAP) decreased from 110±12 to 23±11mmHg (mean±SEM) and from 93±15 to 15±6mmHg in young and old rats, respectively. The difference in the change of MAP between the young and old rats was not significant. With bleeding, mean RNA increased by 46±21% and 20±16% in young and old rats, respectively. The increase in mean RNA of the old rats was significantly lower than that of the young rats. We estimated the gain of the barocepter-renal sympathetic nervous system by using the formula ΔRNA/RNA/ΔMAP/MAP. The gain was 0.61±0.34 and 0.23±0.18 in young and old rats, respectively. The difference in the gain was statistically significant ($p<0.05$). We concluded that the gain of baroceptor-sympathetic nerve system in acute arterial hemorrhage is attenuated with aging.

***Key words***: *Renal nerve activity, Acute arterial bleeding, Aging*

Hypotension and hypovolemia due to acute hemorrhage increase the risk of renal failure with aging, and mortality due to perioperative complications of cardiac and aortic surgery increases with aging[1]. When severe hypovolemic shock occurrs due to rupture of an aneurysm, mortality increases in old human subjects compaired with young. Preventing renal failure is one of the most important problems of hypovolemia, especially in old human subjects. Investigation of the renal response to hypovolemic shock in the young and old is very useful for treatment of old human subjects who fall into hypovolemic shock under anesthesia.

An increase of renal sympathetic nerve activity (RNA) during acute hemorrhage has been reported in anesthetized rats[15], cats[13] and conscious dogs[18]. In anesthetized rats, RNA returns to the pre-hemorrage level after a short-lasting excitation[15]. In conscious dogs[9], RNA decreases to below the pre-hemorrage level after a short excitation. However, the question remains whether the change in RNA during hemorrhage is modified with aging.

Impairment of arterial baroreflexes[7] and cardiopulmonary baroreflexes[8] with age have been reported in dogs. The all reflex arc components may be impaired in old anesthetized rats[16]. On the other hand, the carotid baroreflex function in rats appeared to be well maintained in senescence[17]. The characteristics of carotid sinus baroceptor in old dogs were relatively well maintained compared with those of young dogs[3,4]. The reflex responses of adrenal sympathetic nerve activity to baroceptor stimulation was quite well maintained in rats during aging[10]. The resting activity of the adrenal sympathetic nerve was also at a higher level during aging than in young rats[14]. From these results, we assumed that renal sympathetic nerve activity in response to hemorrhage would be modified with aging.

The aim of this study was to clarify the change in RNA during hypotensive hemorrhage with aging. We compared the change in RNA during severe hemorrhage in young and old rats.

## MATERIALS AND METHODS

*Preparation of animals*

Sixteen male Wistar rats were used in this study. Eight rats were 10 weeks old and their body weight was 280±41 g, and 8 rats were 80 weeks old and body weight was 709±67 g. The rats were anesthetized with sodium pentobarbital (40 mg/kg ip). Additional doses of anesthetic (5 mg/kg) were injected whenever necessary to

maintain surgical anesthesia. Each rat was intubated and spontaneously ventilated with room air. Body temperature was maintained through the experiment by warming. Polyethylene catheters (I.D. 0.6 mm, O.D. 1.0 mm) were canulated from the right common carotid artery to the aortic arch for measuring aortic pressure and from the right carotid vein for injecting fluids and drugs. A third catheter was canulated from the common iliac artery to the abdominal aorta for withdrawing blood.

*Experimental measurements*

Renal sympathetic nerve activity was recorded from the left renal nerve. The left renal plexus was identified behind the renal artery and vein near the hilum of the kidney through the intraperitoneal approach. Then one of the several renal nerves (0.3–0.5 cm) was separated from the plexus, and liquid paraffin was prepared around the nerve. The efferent nerve activity was recorded at the central end of the cut nerve by a pair of Ag-AgCl electrodes. The signal was amplified by a biophysical amplifier (MEG 110, Nihon Kohden, Tokyo, Japan) with a high cut-off frequency of 3000Hz and a low cut-off frequency of 50Hz. This was then passed through a full wave rectifier circuit and integrated with a resistance-capacitance (RC) integrator with a time constant of 20msec. This produced a positive deflection proportional to the frequency discharge in the original neurogram and it was defined as integrated RNA (RNA). Mean RNA (MRNA) was obtained with an RC integrator having a time constant of 1sec. The signals were monitored on a four channel digital storage oscilloscope (VC-6015, Hitachi, Tokyo, Japan).

In all the rats, ECG and aortic pressure (AP) were measured simultaneously. AP was measured with a pressure transducer (DT-MN, Spectramed, Tokyo, Japan).

The original neural signal, RNA, AP, and ECG were displayed on an eight-channel heat-pen recorder (Sanei Retigraph 8K, NEC Sanei, Tokyo, Japan) and stored on a seven-channel magnetic tape recorder (SR-31, TEAC, Tokyo, Japan) for later analysis.

*Experimental protocol and data analysis*

Data was collected under the following conditions:

The preparation was allowed to stabilize for about 30 min, to assure relatively constant mean levels of MRNA and AP. Control data were sampled over a period of 10min. Heparin was administered (500 unit/kg), then the blood (1 ml/100 g body weight) was withdrawn through the catheter with a syringe within 20 sec in young and old rats. The blood was reinfused 5 min after bleeding. Data were sampled continuously during the experiment.

At the end of experiments in all rats, hexamethonium bromide (1 mg/kg, iv) was administered. The majority of RNA was inhibited and decreased to near noise level, thus ensuring that the activity was recorded mainly from a postganglionic sympathetic nerve.

MRNA, AP and heart rate (HR) were recorded simultaneously in young and old rats. The time courses of MRNA, mean AP (MAP) and HR recorded before, during and after 30 sec of bleeding were analysed in young and old rats. MRNA, MAP and HR in the old rats were compared with those in the young before, during and after 30sec of bleeding.

At control before bleeding, MRNA was $9.5 \pm 5.3$ μV in the young rats and $10.3 \pm 6.9$ μV in the old. An absolute value of MRNA is so scattered among different rats that it is too difficult to simply compare during bleeding and hypovolemia. Thus in each rat MRNA at control was set as 100%, and the relative value during bleeding was calculated.

*Statistics*

Data are expressed as mean ±S.E.M. An analysis of variance and Mann-Whitney's test were used. AP value of less than 0.05 was considered to be statistically significant.

## RESULTS

*Young group*

Fig. 1 shows an example of slow speed data of AP, RNA and MRNA recorded from a young rat. With a constant speed of bleeding (0.25 ml/sec), AP decreased progressively in proportion to blood loss volume from 125/100 mmHg to 40/25 mmHg. When AP reached the minimum, AP returned a little, but still decreased significantly. At 20 sec after hypovolemia, AP remained in a hypotension of 50/30 mmHg. MRNA increased rapidly from 8 μV to the maximum level of 14 μV with the start of bleeding. MRNA then decreased gradually during bleeding, and returned to the pre-bleeding level.

MAP, MRNA and HR were measured in eight rats. At control before bleeding, MAP was $110 \pm 12$ mmHg. With bleeding, MAP decreased to a minimum level of $23 \pm 11$ mmHg. The difference in MAP between control and the minimum level was statistically significant. At 30 sec after hypovolemia, MAP increased slightly from the minimum level to $36 \pm 13$ mmHg. The difference in MAP between control and after 30 sec from bleeding was significant (Fig. 2).

During hypovolemia, MRNA increased to a maximum level of $146 \pm 21\%$. The increase in MRNA was statistically significant. At 30sec after hypovolemia, MRNA returned near to the pre-

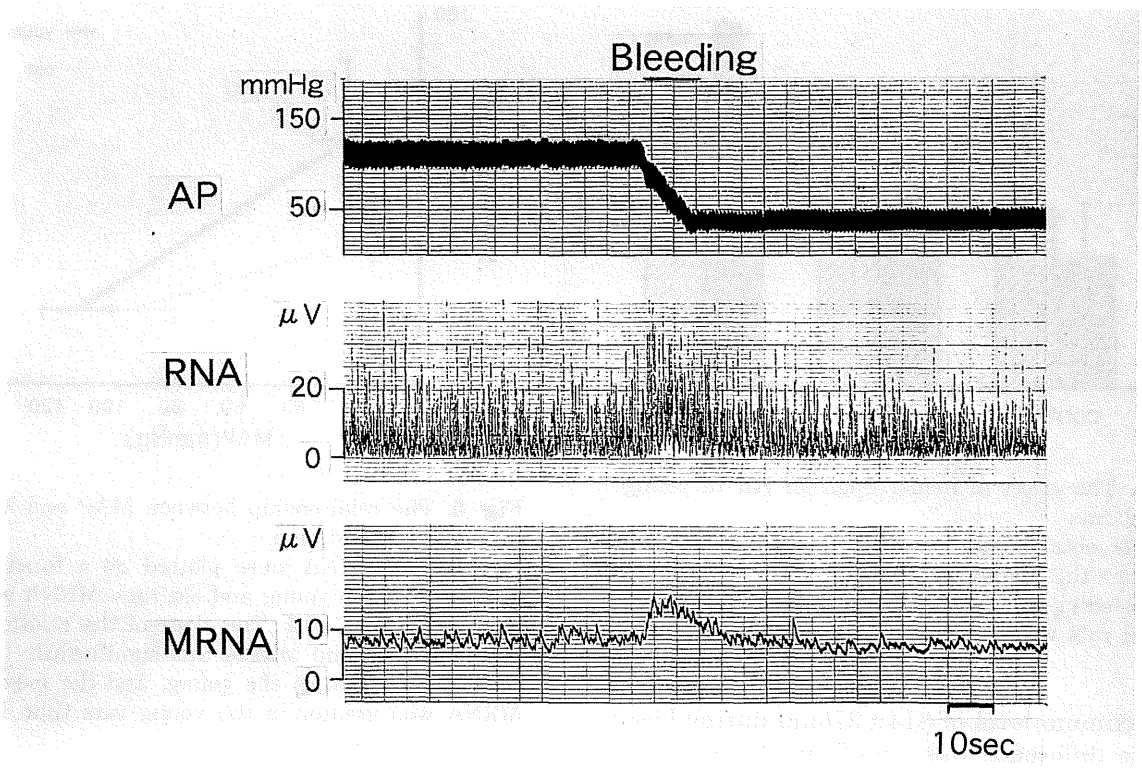


**Fig. 1.** Arterial pressure (AP), renal sympathetic nerve activity (RNA) and mean RNA (MRNA) recorded simultaneously before, during and after acute arterial bleeding in an anesthetized young rat.
With bleeding, AP decreased progressively, while RNA and MRNA increased rapidly. During hypotension and hypovolemia, RNA and MRNA returned near to the control level.

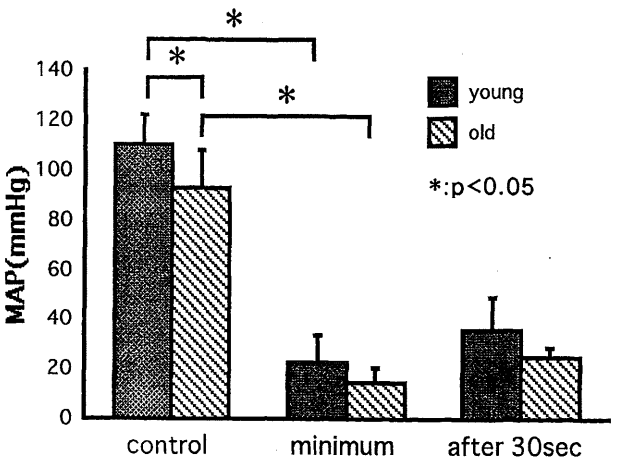


**Fig. 2.** The effect of hemorrhage on mean AP in young and old rats.
Mean AP (MAP) in young rats and old rats were obtained at control, after acute arterial bleeding (the minimum value), and at 30sec from bleeding. MAP decreased significantly in both age groups. The difference in MAP decrease was not significant between young and old rats.

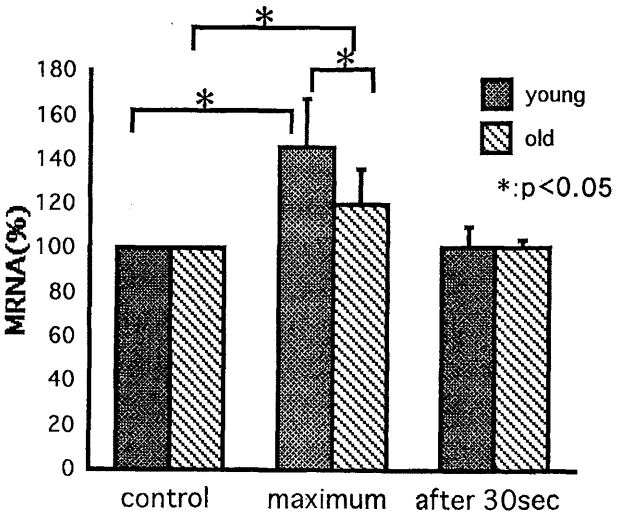


**Fig. 3.** The effect of hemorrhage on MRNA in young and old rats.
MRNA in young rats and old rats were obtained at control, after acute arterial bleeding (the maximum value), and at 30sec from bleeding. MRNA increased significantly in both age groups. The increase in MRNA was significantly greater in the young rats than in the old.

bleeding control level of 101±9%. The difference in MRNA between control and after 30sec was not significant (Fig. 3).

HR was 304±22/min at control and increased

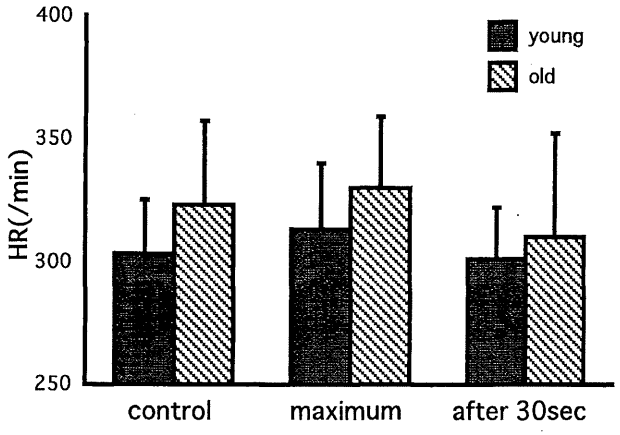


**Fig. 4.** The effect of hemorrhage on HR in young and old rats.
HR was obtained at control, after acute arterial bleeding (the maximum value), and after 30sec from bleeding in young and old rats. HR in young and old rats were not statistically different.

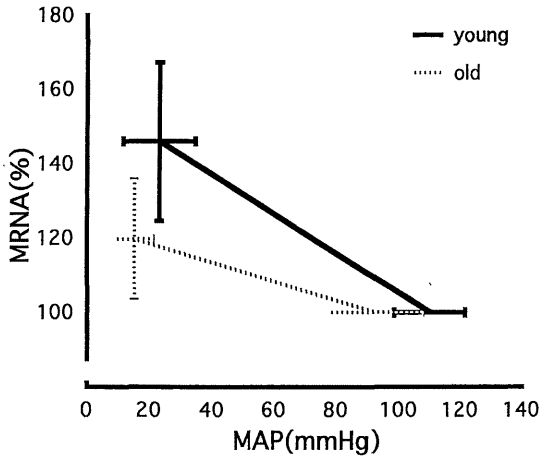


**Fig. 5.** The relationship between MAP and MRNA in young and old rats.
Changes of MRNA were plotted as a function of MAP changes in young and old rats. MRNA at control was set as 100%. The slope of the relationship between MAP and MRNA was significantly less in the old rats than in the young, and the maximum MRNA was greater in the young rats than in the old.

to a maximum level of 314±27/min during bleeding. The difference was significant between control and the maximum level. At 30 sec after hypovolemia, HR was 301±21/min. The difference in HR between control and after 30sec from hypovolemia was not significant (Fig. 4).

*Old group*

MAP, MRNA and HR were measured in eight rats. At control before bleeding, MAP was 93±15 mmHg. MAP decreased significantly to a minimum level of 15±6mmHg. At 30 sec after hypovolemia, MAP increased slightly from the minimum level to 25±4 mmHg. The difference in MAP between control and after 30sec from bleeding was significant (Fig. 2).

During hypovolemia, MRNA increased significantly to a maximum level of 120±16%. At 30sec after hypovolemia, MRNA returned near to the pre-bleeding control level of 101±3%. The difference in MRNA between control and after 30sec was not significant (Fig. 3).

HR was 323±34/min at control and increased significantly to a maximum level of 330±28/min during bleeding. At 30sec after hypovolemia, HR was 301±21/min. The difference in HR between control and after 30sec from hypovolemia was not significant (Fig. 4).

*Comparison of MAP, MRNA and HR between young and old rats*

At control, MAP was significantly higher in the young rats than in the old. The minimum MAP during bleeding and MAP after 30 sec were almost the same in both the young and old rats (Fig. 2).

Absolute MRNA at control was almost the same in young and old rats. The maximum MRNA response to bleeding in the young rats was significantly greater than in the old rats (Fig. 3). However, at 30 sec from hypovolemia, MRNA was almost the same in both age groups.

Although HR in the old rats was slightly greater than that in the young, HR and the change in HR during hypovolemia were not significantly different between young and old rats.

*Relationship between MAP and MRNA during bleeding*

In Fig. 5, the relationship between MAP and MRNA is shown. In young rats, MAP decreased from 110±12 mmHg to 23±11 mmHg, whereas MRNA increased by 46±21%. The slope of the relation line of the young rats was 0.54±0.32 %/mmHg. In the old rats, MAP decreased from 93±15 mmHg to 15±6 mmHg, whereas MRNA increased by 20±16%. The slope of the relation line of the old rats was 0.24±0.22%/mmHg. The slope of the relation line of the young rats was significantly steeper than that of the old rats. To obtain the gain of the baroceptor-renal sympathetic nervous system (i.e. dimensionless value) control MAP was set as 100% in both young and old rats. During bleeding, MAP decreased by 79±10% in the young rats and decreased by 85±6% in the old rats. The decrease in MAP was not significant between young and old rats. We estimated the gain of the baroceptor-renal sympathetic nervous system during bleeding by using the formula Δ MRNA (%)/ΔMAP (%) (Fig. 6). The gain of the young rats was 0.61±0.34. On the other hand, the gain of the old rats was

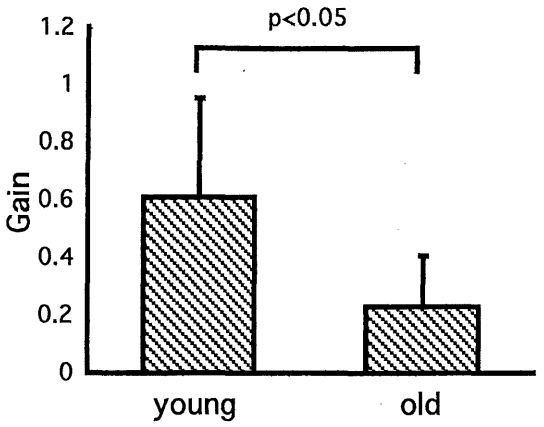


**Fig. 6.** The gain of baroceptor-renal sympathetic nervous system in young and old rats.
The gain was estimated in young and old rats by using the formula ΔMRNA/control MRNA/ΔMAP/control MAP. The gain of the young rats was sigificantly greater than that of the old rats.

$0.23 \pm 0.18$. The gain of the young rats was thus significantly greater than that of the old rats.

## DISCUSSION

In our study, the acute effect of arterial bleeding on AP, MRNA and HR in old rats was compared with that in young rats. MAP significantly decreased whereas MRNA and HR significantly increased in response to acute arterial bleeding (1 ml/100 g) in both age groups. We found that the increase in MRNA per unit change in MAP during bleeding was significantly lower in the old rats than in the young.

*Change in MRNA during acute arterial bleeding*

In our study, MRNA increased with acute arterial bleeding. We assumed that one of the major mechanisms for the increase in MRNA was a baroceptor-reflex in response to hypotension. An increase in sympathetic nerve activity by acute bleeding has already been reported in cats[13], rats[16,17], rabbits[11] and dogs[9,12]. In male Wistar rats (350–400 g), 5 ml/kg bleeding led to a transient increase of RNA, while mean blood pressure decreased by 30 mmHg from 91mmHg[17]. However, quantitative data for the RNA response to bleeding was not shown. In male Wistar-Kyoto rats (300–375 g), after bleeding to 50 mmHg of blood pressure, RNA initially showed a moderate excitation during the first 1–2 min with a maximal increase of 5%[16]. In our study, MAP was decreased to 23 mmHg from 110 mmHg by acute arterial bleeding, while MRNA increased by 46% in the young rats (280 g). The % increase in MRNA in our study was approximately ten times greater than that in previous investigations, since our experiments produced more severe and rapid hypotension. The bleeding volume (i.e. 1 ml/100 g body weight) was sufficient to increase MRNA and to cause hypovolemic shock in the rats.

In our study, the increased MRNA returned near to the control level during hypovolemia after bleeding, although MAP remained in decrease. At 30sec during hypovolemia, MRNA returned to the control level whereas MAP was still significantly lower than at control level. Previous studies showed that MRNA decreased further below control level in rats[17] and dogs[9] during hypovolemia. On the other hand, in anesthetized rabbits, a 10% decrease in blood volume by hemorrhage decreased MAP to $44 \pm 6$ mmHg and caused an increase in MRNA, and after 1–2 min, MRNA remained in increase during hypovolemia[2]. After re-infusion, MRNA returned near to the control level. In the case of a slow hemorrhage of a conscious dog, MRNA increased during non-hypotensive hemorrhage and returned near to the control level during hypotensive hemorrhage[12]. In the case of a slow hemorrhage of a conscious rabbit[11], MRNA increased during non-hypotensive hemorrhage and decreased below control level during hypotensive hemorrhage. During hypovolemia, naloxone was bolus injected and MRNA increased near to the non-hypotensive hemorrhage level[11]. During hypotension due to hypovolemia, MRNA was increased rapidly by the baroceptor reflex mechanism. If the change in MRNA during hemorrhage relates only to the baroceptor reflex, the increase in MRNA during hemorrhage should continue. However, the decrease in MRNA during hypotension and hypovolemia after 30 sec suggests that the baroceptor-reflex system was modified by an inhibition of the central nervous system due to an endogenous system like the secretion of endogenous opiate peptide[11].

*Sympathetic nerve system and aging*

Changes in the sympathetic nerve system with aging have been reported by several investigators. Concerning resting MRNA, there were conflicting reports. Absolute RNA in resting dogs increased with aging[6]. In contrast, in male Wistar rats, absolute RNA in resting decreased with aging[8]. In muscle sympathetic nerve activity in humans, the sympathetic component can be well maintained with advancing age[5]. In our study, absolute MRNA in resting was almost the same in young and old rats. Therefore, relative MRNA in resting was set as 100% in Fig. 5.

Concerning the effect of aging on the baroreflex, there were also conflicting reports. In old dogs, impairment of arterial baroreflexes[6] and cardiopulmonary baroreflexes[7] were observed. In female Sprague-Dawley rats, age-related central and baroceptor impairment was reported[16]. On the other hand, the reflex responses of adrenal

sympathetic nerve activity to stimulation of baroceptors and cutaneous mechanoreceptors in aged rats did not diminish[10]. The maintenance of carotid sinus baroreflex function in advanced age was represented in Fischer rats[18]. In our study, we found that the slope of the relationship between MAP and MRNA was significantly less in old rats than in young.

Therefore, we estimated the gain of the baroceptor-sympathetic nervous system from the relative MAP-MRNA relationship. The gain of the old rats was significantly lower than that of the young rats (Fig. 6). From the data presented in Fig. 5 and 6, we suggest that the baroceptor-sympathetic nervous system was modified with aging.

In conclusion, the baroceptor-renal sympathetic nervous system attenuated with aging and therefore, during acute arterial bleeding, old rats are more prone to hypovolemic shock than young rats.

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