Alterations in the Baroreceptor-heart Rate Reflex in Conscious Inbred Polydipsic (STR/N) Mice

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Summary

STR/N is an inbred strain of mice which is known to exhibit extreme polydipsia and polyuria. We previously found central administration of angiotensin II enhanced cardiovascular responses in STR/N mice than normal mice, suggesting that STR/N mice might exhibit different cardiovascular responses. Therefore, in this study, we investigated daily mean arterial blood pressure and heart rate, and changes in the baroreceptor-heart rate reflex in conscious STR/N mice and control (ICR) mice. We found that variability in daily mean arterial blood pressure and heart rate was significantly larger in STR/N mice than in ICR mice ($p < 0.05$). There was a stronger response to phenylephrine (PE) in STR/N mice than in ICR mice. For baroreceptor reflex sensitivity, in the rapid response period, the slopes of PE and sodium nitroprusside (SNP) were more negative in STR/N mice than in ICR mice. In the later period, the slopes of PE and SNP were negatively correlated between heart rate and blood pressure in ICR mice, but their slopes were positively correlated in STR/N mice. These results indicated that STR/N mice exhibited the different cardiovascular responses than ICR mice, suggesting that the dysfunction of baroreceptor reflex happened in conscious STR/N mice.

Key words

Phenylephrine ● sodium nitroprusside ● baroreceptor reflex ● inbred polydipsic mice

Introduction

The inbred polydipsic mice (STR/N) that was originally developed for studies of gastric neoplasia,

was transferred to the Inbred Colony of the National Institutes of Health in 1951 (Carter et al. 1952),

a genetic abdormality characterized by an extreme polydipsia and polyuria. Unlike polydipsic

Brattleboro rats (Schmale et al. 1984), STR/N strain mice do not possess mutations in the arginine

vasopressin (AVP) and the AVP receptor genes. The AVP mRNA in the hypothalamic

paraventricular nucleus (PVN) and supraoptic nuclei (SON) were increased in STR/N mice than in

control mice (Ison et al. 1993; Yambe et al. 2000).

The angiotensin II (ANG II) stimulates thirst and drinking behavior. In STR/N mice, however, the

responses of neurons of the anteroventral third ventricle region to angiotensin II (ANG II) are

significantly attenuated (Hattori and Koizumi 1990) and ANG II receptor binding is abundant in the

brain stem region, particularly in the nucleus tractus solitarius (NTS) (Tribollet et al. 2002).

Substantial evidence has been accumulated suggesting that circulating vasoactive peptides, such as

ANG II and AVP, act at the central nervous system to modulate sympathetic and vagal activity, and

ultimately regulate baroreflex sensitivity (BRS) (Bishop and Hay 1993; Hasser et al. 1997; Bishop

and Sanderford 2000).

The arterial baroreflex is an important determinant of neural regulation of the cardiovascular

system. Baroreflex-mediated sympathoexcitation contributes to the development and progression of

many cardiovascular disorders. Accordingly, the quantitative estimation of the arterial baroreceptor-heart rate reflex has been regarded as a synthetic index of neural regulation at the sinus atrial node. Evaluation of BRS provides clinical and prognostic information in a variety of cardiovascular diseases (La Rovere et al. 2013). In normal physiological conditions, changes in arterial pressure affect sympathetic nerve activity via the neural arc, and changes in sympathetic nerve activity, in turn, affect arterial pressure via the peripheral arc. The vasoactive peptides ANG II and AVP are thought to be involved in the modulation of baroreflex control of heart rate by central sites, such as the area postrema and the nucleus tractus solitarius (NTS) (Cox et al. 1990; Matsukawa et al. 1990; Peuler et al. 1990). On the other hand, some reports indicate that a chronic hypotension might cause polydipsia because of an inhibitory influence of arterial baroreceptors on water intake (Rettig and Johnson 1986; Robinson and Evered 1987). We found arterial blood pressure of the polydipsic mice was lower than control mice (Chu et al. 2003), and enhanced effects of central exogenous ANG II on cardiovascular and drinking responses in inbred polydipsic (STR/N) mice. Thus the polydipsia is likely to be related to baroreceptor activity in STR/N mice. This remains to be determined.

Taken together, these findings suggest that BRS may be changed in STR/N mice. However, this remains to be determined. Therefore, the objective of the current study was to investigate changes in BRS of STR/N mice by examining cardiovascular parameters and responses of vasoactive drugs.

Materials and Methods

Animals

Male inbred polydipsic STR/N strain mice and ICR (Institute of Cancer Research, also known as Swiss-Webster, controls) mice at 16–18 weeks of age were used. All experiments were approved by the Committee on Animal Care of Miyazaki Medical College. STR/N and ICR mice were kept in the Miyazaki Medical College Experimental Animal Center under controlled conditions, at a temperature of $23 \pm 2°C$ and humidity of $60\% \pm 10\%$. Animals were fed standard mouse chow and had access to tap water *ad libitum*.

Animal preparation and data collection

Mice were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). Supplemental doses of anesthetics (1/5 of the initial dose) were administered as required to prevent eye blinking and withdrawal reflexes (Chu et al. 2003). The femoral artery and vein were exposed, and PE-10 tubing heat-coupled to PE-50 tubing of two catheters were filled with heparinized (100 U/ml) saline. These were inserted into the femoral artery and vein for arterial BP measurement and drug injections, respectively. The catheters were then tunneled subcutaneously, exteriorized, and placed at the back of the neck. The arterial catheter was connected to a BP transducer (Gould, Saddle Brook, NJ, USA) placed in a horizontal position level with the heart to monitor arterial BP. Heart rate (HR) was measured using a cardiotachometer (AT601G, Nihon Kohden, Tokyo, Japan) triggered by the rising

portion of arterial BP. Signals were sent to data acquisition systems (Intelligent Instrumentation, Inc., Tucson, AZ, USA) for the calculation of mean arterial blood pressure (MABP) and HR. Mice were put into a custom-made special cage for recovery and experimental recording.

Twenty-four-hour BP recording

All experiments were performed on conscious, freely moving mice, 3 days after surgery. The STR/N and ICR mice were maintained on a 12-h light/dark cycle, and received food and water *ad libitum*. BP and HR were recorded continuously for 24 h using computer systems for 1 day, starting in the morning at 06:00 to the next morning at 06:00.

Evaluation of baroreflex control of HR

All experiments were performed on conscious, freely moving mice, 3 days after surgery. Baroreflex control of HR was determined by recording reflex changes in HR in response to transient increases in MABP produced by bolus injections of graded doses of phenylephrine (PE, stocking solusion is 2.5 μg/25 μL, 10 μg/kg, 25 μg/kg and 35 μg/kg, Sigma), or decreases in MABP induced by sodium nitroprusside (SNP, stocking solusion is 2.5 μg/25 μL, 5 μg/kg, 15 μg/kg and 25 μg/kg, Sigma) into the femoral vein. At the end of injections (up to 15 μl/mouse), catheters were immediately flushed with 35 μl saline at 250 μl/min (by a pump, kdScientific, USA). BP and HR returned to basal levels before the next dose was given. To investigate changes in the correlation of BP and HR in full time, the data were divided into two time periods according to the mean values of

the time courses of every dose. The rapid response period was determined from the baseline to the

peak (SNP) or nadir (PE) of the HR response to SNP or PE (rectangular box shown in Figs. 1, 2).

The later period was defined as the time after the rapid response period to HR recovery. The best fit

linear relationship between reflex changes in HR and changes in MABP was plotted and the slope

was calculated.

Statistical analysis

All data are expressed as means ± SE. Statistical analyses were performed using ANOVA.

Maximum changes from control values were analyzed using the Student's t-test. To analyze

baroreflex responses, HR values during PE or SNP were plotted against the corresponding MABP

values for each mouse, and the data were subjected to linear regression analysis. A value of $p < 0.05$

was considered statistically significant.

Results

Basal BP and HR

MABP and HR were measured 3 days after the surgery in STR/N and ICR mice in a conscious,

freely moving state (Table 1). Twenty-four-hour MABP was 87.2 ± 3.5 mmHg in STR/N mice and

108.7 ± 2.5 mmHg in ICR mice. MABP of STR/N mice was significantly lower than that of ICR

mice ($p < 0.01$), but mean HR was similar in both strains of mice (537.5 ± 11.1 and 553.0 ± 26.4

beats/min in STR/N and ICR mice, respectively). During 24 h, the peak and nadir of BP were

significantly lower in STR/N mice than in ICR mice ($p < 0.05$). However, the peak daily HR was significantly higher in STR/N mice than in ICR mice ($p < 0.05$) and the nadir of daily HR was significantly lower in STR/N mice than in ICR mice ($p < 0.05$). The absolute changes in MABP ($p < 0.05$) and HR ($p < 0.05$) over 24 h in STR/N mice were significantly higher than those in ICR mice.

Cardiovascular responses to i.v. injection of PE

Intravenous injection of PE (25 μg/kg, Figs. 1) provoked an increase in MABP (STR/N: 87.8 ± 1.7 to 139.5 ± 12.6 mmHg, ICR: 105.3 ± 1.6 to 134.2 ± 3.1 mmHg) and a decrease in HR (STR/N: 530.9 ± 6.0 to 262.9 ± 58.2 beats/min, ICR: 547.7 ± 8.5 to 432.5 ± 25.4 beats/min) in both groups. MABP and HR recovered after approximately 40 s in ICR mice. PE caused a decrease in HR in STR/N mice, and this recovered after approximately 40 s. However, the duration of BP response to PE in STR/N mice (> 1200 s) was significantly longer than that in ICR mice ($p < 0.05$). The absolute increase in MABP in STR/N mice (51.3 ± 7.7 mmHg) was significantly larger than that in ICR mice (28.7 ± 2.4 mmHg, $p < 0.05$). The absolute decrease in HR in STR/N mice (262.8 ± 42.4 beats/min) was significantly higher than that in ICR mice (112.7 ± 14.9 beats/min, $p < 0.01$).

Cardiovascular responses to i.v. injection of SNP

Intravenous injection of SNP (25 μg/kg, Figs. 2) induced a decrease in MABP (STR/N: 84.1 ± 2.1 to 34.6 ± 5.1 mmHg, ICR: 103.8 ± 2.5 to 52.2 ± 3.4 mmHg) and an increase in HR (STR/N: 535.3 ± 8.2 to 589.8 ± 10.1 beats/min, ICR: 535.7 ± 10.5 to 583.3 ± 14.8 beats/min). The decrease in MABP

caused by SNP in STR/N mice recovered after 50 s. However, the duration of the HR response to

SNP in STR/N mice was unstable within approximately 80 s. The absolute decrease in MABP (49.3

± 2.7 mmHg) in STR/N mice was similar to that in ICR mice (50.8 ± 3.0 mmHg, $p > 0.05$). The

absolute increase in HR (51.5 ± 8.0 beats/min) in STR/N mice was similar to that in ICR mice (46.4

± 9.1 beats/min, $p > 0.05$).

Baroreflex response to PE and SNP

In the rapid response period, ANOVA of the linear regression showed a significant dependence of

HR on BP in ICR and STR/N mice. There was also a negative correlation between HR and BP in

response to PE or SNP in both strains of mice (Table 2 and Fig. 3). For statistical analysis in which

data from all observations were pooled, slopes for the response to PE or SNP were significantly

more negative for STR/N mice (slope = -9.22 ± 0.72 with PE; -2.16 ± 0.18 with SNP) than for ICR

mice (slope = -3.98 ± 0.75 with PE; -0.97 ± 0.05 with SNP) ($p < 0.05$, Table 2). In the later period,

ANOVA of linear regression showed a significant dependence of HR on BP in response to PE or

SNP for ICR and STR/N mice. There was a negative correlation between HR and BP in response to

PE or SNP, and no significant difference between the rapid response and later period in ICR mice

(Table 2). However, there was a negative correlation between HR and BP in the rapid response

period and a positive correlation in the later period in response to PE or SNP in STR/N mice (slope =

4.09 ± 1.27 with PE; 0.37 ± 0.08 with SNP). There was a significant difference between the rapid

response and later periods in STR/N mice (p < 0.05) (Table 2 and Fig. 3).

Discussion

The present study found that in conscious mice 1) the absolute change in MABP and HR values over 24 h was significantly larger in STR/N mice than in ICR mice, 2) there was a stronger response to PE in STR/N mice than in ICR mice, and 3) with regard to BRS, in the rapid response period, the slopes of PE and SNP were more negative in STR/N mice than in ICR mice. In the later period, the slopes of PE and SNP changed to a positive correlation between HR and BP in STR/N mice. These data demonstrate that baroreflex activity is altered in STR/N mice, particularly in the later period, where the negative correlation between BP and HR disappears.

The present study showed that the absolute change in daily MABP and HR in STR/N mice was significantly higher than that in ICR mice (Table 1). The arterial baroreceptor reflex system plays a predominant role in preventing short-term wide fluctuations of arterial BP in many animal species. Arterial baroreceptor denervation results in an increase in variability of BP (Cowley et al. 1973). Basic pathways mediating reflex control of HR involve baroreceptors, afferents to the central nervous system, the cardiovascular center in the central nervous system, and sympathetic and parasympathetic efferents to the heart (Kumada et al. 1990). Impairment at any point along these pathways leads to an altered baroreflex. While the mechanisms for this process are still unclear, the renin–angiotensin system and other humoral and/or hormonal factors may be involved (Lohmeier

2001; Nishida et al. 2012). STR/N mice have different central ANG II and AVP systems compared

with controls (Nishi et al. 1999; Hattori and Koizumi 1990; Tribollet et al. 2002; Chu et al. 2003).

The instability of BP and HR implies impairment of the baroreflex in STR/N mice. In addition,

prolonged stimulation of the baroreceptors can produce decreases in BP that last for up to 1 week

(Lohmeier et al. 2005). Such data support the hypothesis that the baroreflex is not only important in

moment-to-moment regulation, but is also involved in long-term regulation, and therefore may

influence more chronic maladaptations. A low BP in STR/N mice has been previously reported by

our group (Chu et al. 2003). One possible explanation for the lower basal BP in STR/N mice

compared with controls in the present study is the occurrence of an increased gain of the baroreflex.

Our results indicated that in the rapid response period, the slopes of PE and SNP were more negative

in STR/N mice than in ICR mice (Fig. 3 and Table 2). Holschneider et al. (2002) reported that the

baroreceptor response was increased in MAO-A/B KO mice with evident lower BP. Under

normotensive conditions, baroreceptor input tonically inhibits sympathetic effects on blood vessels

and the heart. An increased gain of the baroreflex could lead to lower BP in response to the

excessive basal levels of pressor amines. Results of the present study are consistent with this

possibility. However, we cannot exclude the possibility that an altered set point may induce lower

basal BP, which can occur through serotonergic mechanisms thought to be independent of the

baroreceptor reflex (Nosjean et al. 1995; Tsukamoto et al. 2000). Yambe et al. (2000) reported that

dehydration occurred with the acceleration of AVP synthesis because of deterioration of AVP sensitivity in 20-week-old STR/N mice. Therefore, it is conceivable that relative hypovolemia secondary to increased urine output in STR/N mice might result in decreased BP.

This study indicated that there was a stronger response to PE in STR/N mice than in ICR mice (Fig. 1). PE is an α1-adrenergic agonist. Masuki et al. (2003; 2005) reported that enhanced baroreflex control of HR might compensate for blunted peripheral α-adrenergic responsiveness in blood vessels, helping to maintain the stability of arterial BP. Our results showed that baroreflex control of HR and peripheral α-adrenergic responsiveness in blood vessels were enhanced in STR/N mice compared with ICR mice (Figs. 1, 3A). Although the abnormity of the α-adrenergic system has not been reported, as mentioned above, STR/N mice have a different central ANG II system compared with controls (Hattori and Koizumi 1990; Tribollet et al. 2002; Chu et al. 2003). Cross-talk between angiotensin type 1 receptor and α1-adrenergic receptors has been reported and indicates the existence of physiological regulation between the renin–angiotensin system and the sympathetic nervous system at the receptor level in the brain (Crespo 2000; Stadler et al. 1992). There is endothelium-dependent cross-talk between α1-adrenergic and ANG II receptors in smooth muscle of the rabbit aorta (Jerez et al. 2004). These results suggest that the adrenergic system might be changed in STR/N mice. Future studies will be required to determine this issue.

Recently, some reports have suggested that BRS is reduced during hypertension and increased

during hypotension (Braga 2010; Botelho-Ono et al. 2011; Guimarães et al. 2012; Queiroz et al. 2012; Holschneider et al. 2002). This finding is consistent with our results. Previously, we reported a lower BP in STR/N mice (Chu et al. 2003). The present study showed that in the baroreflex response to PE and SNP, the slopes were more negative in STR/N mice than in ICR mice in the rapid response period (Table 2). This indicated an increased gain of the baroreflex in STR/N mice. Nishi et al. (1999) reported that AVP mRNA levels in the hypothalamus were higher in polydipsic STR/N mice than in control ICR mice. Transgenic animal research has shown that V1a$^{-/-}$ mice (Koshimizu et al. 2006) and AVP-deficient mutant Brattleboro rats have attenuated BRS (Imai et al. 1983 a and 1983b). AVP has been shown to enhance baroreflex function via activation of V1 receptors in the area postrema (Hasser and Bishop 1990; Scheuer and Bishop 1996; Cox et al. 1990). Circulating AVP increases the gain or sensitivity of the baroreflex through its action in the area postrema (Cowley and Barber 1983; Hasser et al. 1997). This might be a reason for enhanced BRS in STR/N mice. The mechanisms of the alteration of baroreflex activity in STR/N mice need to be studied in the future.

A key component of baroreceptor reflex is the baroreceptor-HR reflex which induces rapid HR responses to alterations in BP. Under normal condition, activation of arterial baroreceptors by a rise in systemic arterial pressure leads to an increase of the discharge of vagal cardioinhibitory neurons and decrease in the discharge of sympathetic neurons both to decrease HR. Conversely, a decrease in

systemic arterial pressure causes the deactivation of baroreceptors with subsequent enhancement of

sympathetic activity and vagal inhibition, leading to tachycardia (La Rovere et al. 2008). In the

present study, we found that BP and HR gradually returned to normal follow the baroreceptor reflex

principle at the end of the potency of PE and SNP in ICR mice. However, the correlations between

MABP and HR were reversed in the later period of the baroreceptor reflex in STR/N mice. This kind

of alteration might be due to changes in AVP, ANG II, or adrenergic systems in STR/N mice. It is

remained unclear.

In conclusion, the present study indicated that STR/N mice exhibited the different cardiovascular

responses than ICR mice, suggesting that the dysfunction of baroreceptor reflex happened in

conscious STR/N mice.

Acknowledgements

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Table1. Daily basal parameters of MABP and HR in conscious ICR and SRT/N mice.

	Animal	n	Mean	tiptop	nadir	change
MABP (mmHg)	ICR	9	108.7±2.5	113.6±2.4	100.4±1.8	13.2±1.6
	SRT/N	9	87.2±3.5**	97.8±2.7*	70.4±1.7*	27.3±1.9*
HR (bpm)	ICR	9	553.0±26.4	597.1±28.9	506.4±25.2	91.3±22.3
	SRT/N	9	537.5±11.1	640.5±18.7*	451.2±21.6*	189.3±27.2*

Values are expressed as mean ± SE; n is no. of animals. * $P<0.05$ vs. ICR. ** $P<0.01$ vs. ICR.

Table 2. Slopes of Baroreflex responses to PE and SNP in ICR and STR/N mice.

	ICR			STR/N		
	Slope	F values	P values	Slope	F values	P values
PE						
Rapid response period	-3.98±0.75	27.87	<0.005	-9.22±0.72*	164.55	<0.000005
Later period	-3.66±0.25	207.38	<0.000005	4.09±1.27*#	10.42	<0.005
SNP						
Rapid response period	-0.97±0.05	435.80	<0.000005	-2.16±0.18*	144.92	<0.0005
Later period	-1.13±0.07	274.29	<0.000005	0.37±0.08*#	20.76	<0.00005

Values are means ± SE; n = 6 male mice. PE, phenylephrine; SNP, sodium nitroprusside. *P<0.05 vs.

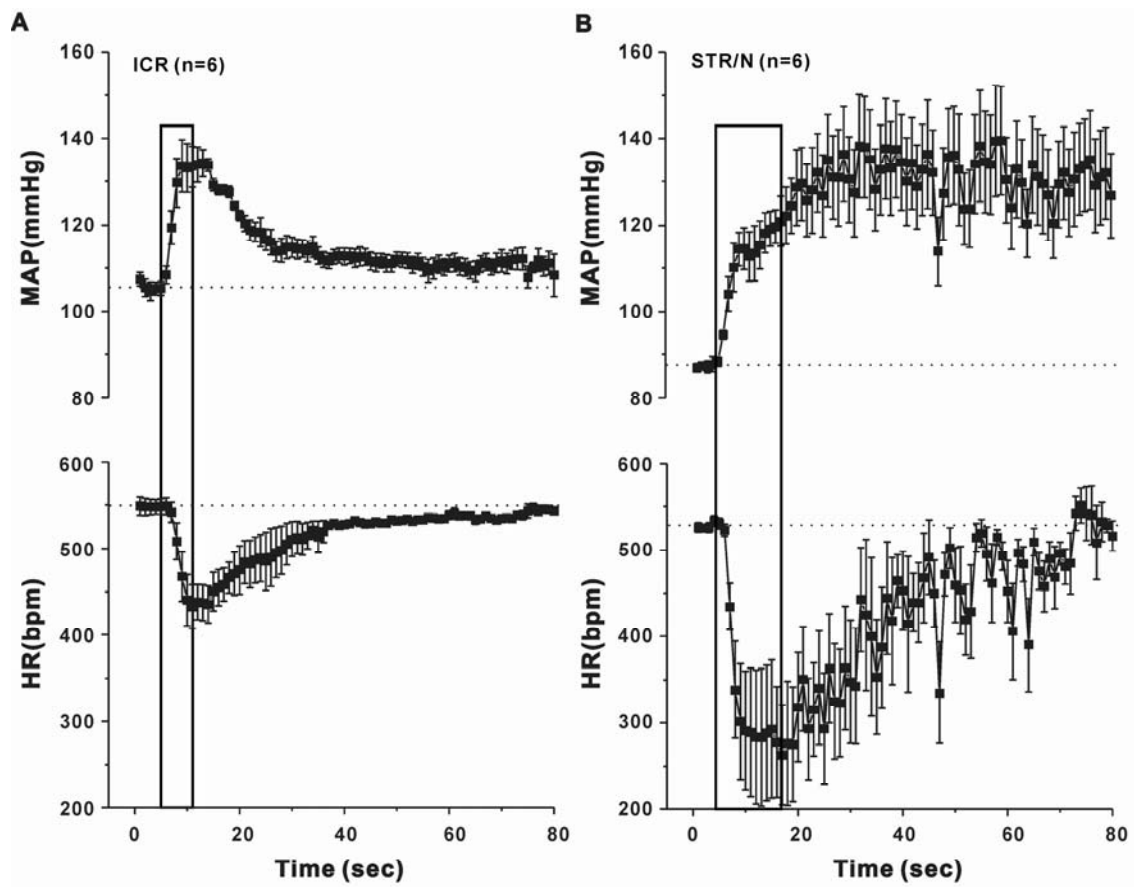ICR. #P<0.05 later period vs. rapid response period.

Fig. 1. Time course of changes in mean arterial blood pressure (MABP) and heart rate (HR) after i.v. administration of PE (25 μg/kg) in conscious mice. A: ICR mice; B: STR/N mice. The rectangular box indicates the rapid response period; bpm, beats/min; n, number of animals. All data are means ± SE.
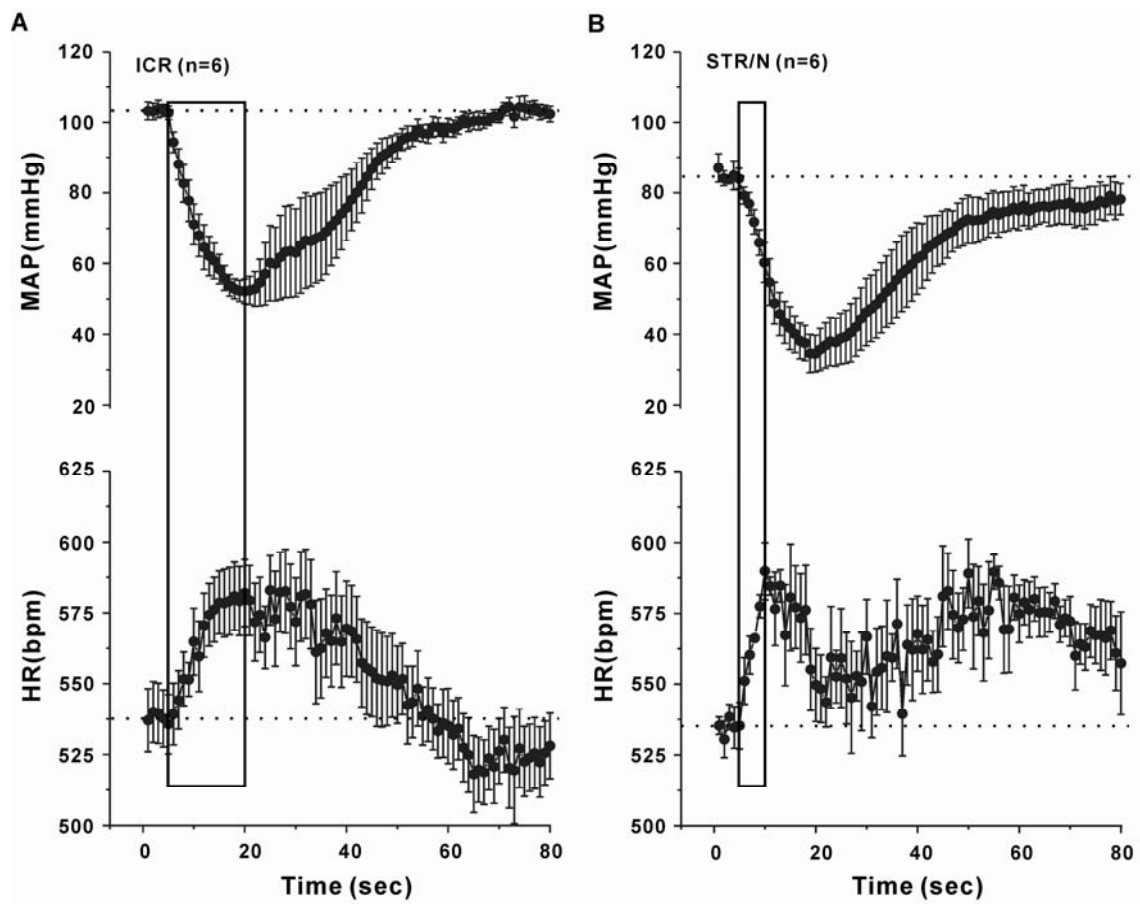
Fig. 2. Time course of changes in mean arterial blood pressure (MABP) and heart rate (HR) after i.v. administration of SNP (25 μg/kg) in conscious mice. A: ICR mice; B: STR/N mice. The rectangular box indicates the rapid response period; bpm, beats/min; n, number of animals. All data are means ± SE.
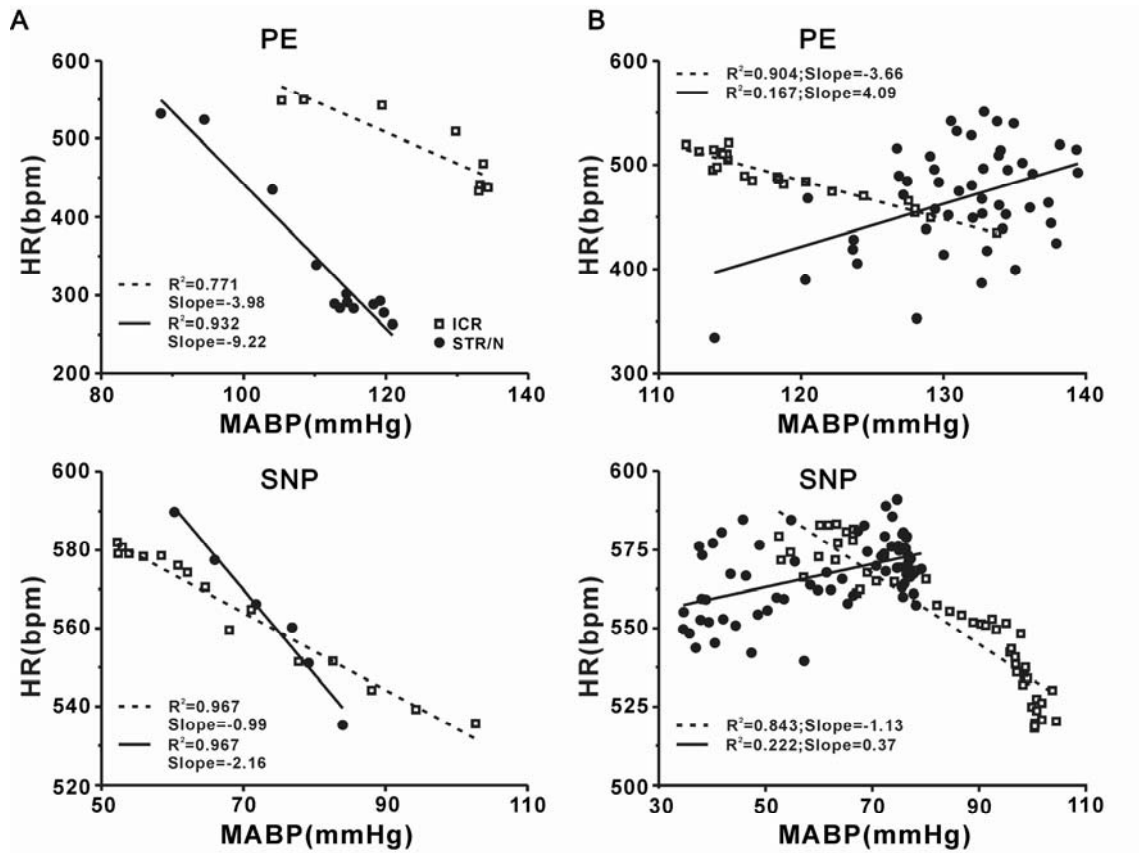
Fig. 3. Baroreflex sensitivity. A: Regression lines for the association between HR and MABP at the rapid response period (rectangular box shown in Figs. 1, 2) for PE (top) and SNP (bottom) in STR/N and ICR mice. B: Regression lines for the association between HR and MABP at the later period (period of time after the response period) for PE (top) and SNP (bottom) in STR/N and ICR mice. Dashed lines are the average slopes of the regression lines in ICR mice. Thick lines are the average slopes of the regression lines in STR/N mice. n = 6.