Aging Escalates Baroreceptor Reflex Suppression by the Posterior Hypothalamus in Rats

Shinichi Tanabe and Ruben D. Bunag

To examine whether baroreceptor reflex regulation by the posterior hypothalamus becomes modified with age, we compared baroreceptor reflex sensitivity and hypothalamic responsiveness in 2- and 10-month-old rats anesthetized with urethane-chloralose. Hypothalamic regulation of baroreceptor reflex sensitivity was assessed by recording responses to intravenously infused phenylephrine and afferent aortic nerve stimulation after sham operation or electrolytic destruction of the posterior hypothalamus. Regardless of age, reflex bradycardia and sympathoinhibition elicited during pressor responses to phenylephrine, as well as all cardiovascular and sympathetic nerve responses to afferent aortic nerve stimulation, were stronger in rats with bilateral hypothalamic lesions than in age-matched, sham-operated controls. Distinctively, because baroreceptor reflex sensitivity differed with age only in sham-operated controls but not in lesioned rats, we concluded that age-related differences in baroreceptor reflex sensitivity had been abolished by posterior hypothalamic lesions. Other experiments were then performed to compare responses to graded electrical stimulation of the posterior hypothalamus in baroreceptor-intact rats. Pressor and sympathoexcitatory responses to hypothalamic stimulation were larger, and stimulus thresholds were lower at 10 than at 2 months of age thereby suggesting that hypothalamic responsiveness had increased with age. Our results are in accord with the interpretation that aging exacerbates the baroreceptor reflex suppression normally exerted by the posterior hypothalamus. (Hypertension 1991;17:80-90)

Blood pressure tends to rise with age and adrenergic cardiac regulation and neurotransmission decline until cardiovascular homeostasis eventually fails to possibly facilitate development of hypertension. Because baroreceptor reflex buffering normally plays a major role in maintaining cardiovascular homeostasis, it seems logical to anticipate that baroreceptor reflex sensitivity would diminish with age. An age-related baroreceptor reflex impairment in conscious rats was first described by Rothbaum et al, who showed that phenylephrine produced less reflex bradycardia in 24-month-old rats than in 12-month-old rats. More recently, on comparing 9- and 2-month-old rats we surmised that the sites affected on the reflex arc may be central because we found that reflex responses to afferent aortic nerve stimulation were likewise reduced with age.4

The idea that the posterior hypothalamus participates in baroreceptor reflex regulation stems from the original observation made in 1956 by Gellhorn et al that posterior hypothalamic lesions enhance reflex bradycardia. Since then, many have used electrical stimulation (instead of lesions) of the posterior hypothalamus to suppress reflex vagal bradycardia. Although the responses thus obtained could result from activation of fibers originating from other brain areas, the finding that posterior hypothalamic stimulation inhibits reflex bradycardia has nevertheless been confirmed by using chemical stimuli, which affect cell bodies but not fibers of passage.8

Considering the changes in hypothalamic function that normally occur with increasing age, it now seems reasonable to speculate that the age-related baroreceptor reflex impairment3,4 may be due, at least in part, to concurrent alterations in hypothalamic activity. To explore this possibility, we initially aimed to determine whether electrolytic destruction of the posterior hypothalamus would affect barore-
receptor reflex sensitivity similarly in 2- and 10-month-old rats. Baroreceptor reflex sensitivity was quantified by recording responses to injected phenylephrine or afferent aortic nerve stimulation. Because reflex responses to both stimuli were significantly enhanced by hypothalamic destruction regardless of rat age, additional experiments were done to compare hypothalamic responsiveness by recording cardiovascular and sympathetic responses to graded electrical stimulation of the posterior hypothalamus.

Methods

Rats Groups and General Procedure

Male rats purchased from Harlan Sprague Dawley, Indianapolis, Ind., were studied at 2 months old, weighing 170–370 g and at 10 months old, weighing 275–540 g. Each rat was first anesthetized with methoxyflurane for surgical implantation of indwelling vascular cannulae to be used for injecting drugs and recording blood pressure. Further anesthesia was then induced with urethane-chloralose so that terminal experiments could be done to assess baroreceptor reflex sensitivity and hypothalamic responsiveness. Two groups of terminal experiments were performed to record: 1) baroreceptor reflex sensitivity henceforth defined to include responses to intravenous infusion of phenylephrine or electrical stimulation of the left aortic depressor nerve in sham-operated or lesioned rats in group 1 and 2) hypothalamic responsiveness consisting of responses and thresholds to graded electrical stimulation of the posterior hypothalamus in intact rats in group 2. Although 92 rats were used altogether, data from 22 rats had to be discarded because of technical difficulties with anesthesia, hemorrhage, or nerve damage; consequently, results finally analyzed were from 70 (35 in each age group) rats.

Anesthesia and Surgical Cannulation

Separate indwelling cannulae were surgically inserted into a femoral artery for recording blood pressure and a femoral vein for infusing drugs while the rats were anesthetized with methoxyflurane (meto- fane by inhalation). Each cannula consisted of two pieces of Teflon (26 gauge, i.d. 0.018 in., 4 cm long, Small Parts Inc., Miami, Fla.) and Tygon (i.d. 0.04 in., o.d. 0.07 in., 15 cm long, Norton Co., Akron, Ohio) tubing bonded together with cyanoacrylate adhesive (Wonder Bond Plus, Borden Inc., Columbus, Ohio). The free end of the smaller bore Teflon tubing was inserted into the blood vessel being cannulated while that of the larger bore Tygon tubing was connected to a pressure transducer or syringe pump. During subsequent terminal experiments, because older rats were larger than young ones, further anesthesia was equalized by using comparable anesthetic doses expressed in terms of 100 g body wt: urethane (20 mg/100 g in group 1 and 45 mg/100 g in group 2) was injected through the femoral vein cannula followed with α-chloralose (5 mg/100 g with equal portions injected subcutaneously and intraperitoneally).

Cardiovascular Variables and Splanchnic Nerve Recording

Pulsatile arterial pressure was recorded by connecting the femoral artery cannula through thick-walled Tygon tubing (19 gauge, type S-54-HL) to a small volume-displacement pressure transducer (MP-15, Micron Instruments Inc., Los Angeles, Calif.) placed on the same level as the rat. Heart rates were monitored simultaneously by triggering a biotachometer with the phasic pressure signal from the transducer.

To record sympathetic nerve activity, the splanchnic nerve (identified as the major nerve bundle entering the coeliac plexus) was exposed through a ventral transabdominal incision. With the aid of a dissecting microscope, a 5–7 mm length of the nerve was carefully separated from surrounding tissues and placed over a bipolar platinum electrode ( uninsulated tips about 2 mm apart) connected to a probe from the differential amplifier. Tissue drying was prevented by filling the abdominal cavity with enough mineral oil to immerse the exposed nerve together with the electrode tips. During each experiment, spike potentials were amplified (DAM 80 AC differential amplifier, World Precision Instruments, New Haven, Conn.) and recorded continuously on magnetic tapes as described previously. These tapes were later played back into an amplitude analyzer (Frederick Haer and Co., Brunswick, Me.) to convert individual spikes into uniform pulses and the number of individual pulses per unit of time (1 or 2 seconds) was counted with a rate analyzer whose output was recorded as a histogram.

Group 1 Terminal Experiments: Effects of Bilateral Electrolytic Lesions of the Posterior Hypothalamus on Baroreceptor Reflex Sensitivity

A coaxial electrode (NE-100) was inserted into the posterior hypothalamus alternately on each side of the brain using the same stereotaxic coordinates mentioned below while the rats were anesthetized with pentobarbital (3.5 mg/100 g). Electrolytic lesions were made by passing a 1-mA DC current for 10 seconds while sham-operations consisted only of electrode insertion but without current passage. Terminal experiments were done after allowing about 1 week for postoperative recovery.

To assess effects of hypothalamic destruction on baroreceptor reflex sensitivity, phasic blood pressure, heart rate, and splanchnic nerve activity were recorded continuously as blood pressure was elevated by intravenous infusion of phenylephrine. By infusion solutions containing 20 mg phenylephrine/100 ml at an initial rate of 3 μl/100 g with 0.57 μl/100 g increments every 4 seconds, progressive blood pressure increases up to 50 mm Hg in 1–2 minutes were attained. All infusions were delivered using a 1 ml syringe mounted on a syringe pump (model 22, Harvard Apparatus, South Natick, Mass.) driven by a computer (TRS 80 model III, Radio Shack) pro-
Hypothalamic Responsiveness and Thresholds to while blood pressure, heart rate, and splanchnic

Grammed to alter infusion rates by increasing number and duration of stepwise increments based on the rat's body weight.

Subsequently, cardiovascular and sympathetic nerve responses to graded electrical stimulation of the left afferent aortic nerve were also recorded in the same rats. After cutting the right aortic nerve and the carotid sinus, laryngeal and glossopharyngeal nerves on both sides, the left aortic nerve was sectioned as low as possible in the neck and its central cut end was placed on a bipolar platinum electrode (as described above). A square-wave stimulator (Grass S88 with PSIU6 isolation unit, Grass Instrument Co., Quincy, Mass.) was then used to stimulate the left afferent aortic nerve for 30 seconds (with 3-mA pulses lasting for 1 msec at frequencies from 1 to 16 Hz) while blood pressure, heart rate, and splanchnic nerve activity were recorded continuously.

Group 2 Terminal Experiments: Posterior Hypothalamic Responsiveness and Thresholds to Graded Electrical Stimulation in Intact Rats

After positioning the rat's head in the stereotaxic apparatus (David Kopf Instruments, Tujunga, Calif.) with the tooth bar placed at a plane 5.0 mm above the ear bars, a coaxial electrode (NE-100, center contact diameter 0.2 mm, Rhodes Medical Instruments, Woodland Hills, Calif.) was inserted using standard stereotaxic coordinates: anteroposterior 4.6, left lateral 1.0, and dorsoventral -2.5. A square-wave stimulator (Grass S88 with PSIU6 isolation unit) was used to deliver 10-second current trains ranging in strength from 50 to 300 µA (pulse duration 1 msec, frequency 100/sec) to the hypothalamic electrode while blood pressure, heart rate, and splanchnic nerve activity were continuously recorded. As previously described, current thresholds were determined in each rat by starting stimulation with 50 µA and progressively increasing current strength by 20 µA increments until a 2 mm Hg increase in mean pressure occurred.

### Table 1. Reflex Bradycardia and Sympathetic Nerve Inhibition During Intravenous Infusions of Phenylephrine in Rats Anesthetized With Urethane-Chloralose

<table>
<thead>
<tr>
<th>Rat groups</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reflex bradycardia (beats/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-mo, sham</td>
<td>-8±1</td>
<td>-10±1</td>
<td>-16±1</td>
<td>-22±2</td>
<td>-29±2</td>
</tr>
<tr>
<td>2-mo, lesioned*</td>
<td>-5±1</td>
<td>-12±2</td>
<td>-20±3</td>
<td>-33±5†</td>
<td>-37±4</td>
</tr>
<tr>
<td>10-mo, sham‡</td>
<td>-4±1</td>
<td>-7±2</td>
<td>-12±2</td>
<td>-15±2</td>
<td>-20±2</td>
</tr>
<tr>
<td>10-mo, lesioned*</td>
<td>-5±1</td>
<td>-10±2</td>
<td>-19±3</td>
<td>-29±4†</td>
<td>-34±4†</td>
</tr>
<tr>
<td>F ratio</td>
<td>11.49</td>
<td></td>
<td></td>
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</table>

Reflex sympathetic inhibition (% change)

<table>
<thead>
<tr>
<th>Rat groups</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-mo, sham</td>
<td>-29±3</td>
<td>-40±4</td>
<td>-50±4</td>
<td>-52±4</td>
<td>-53±4</td>
</tr>
<tr>
<td>2-mo, lesioned*</td>
<td>-37±4</td>
<td>-47±4</td>
<td>-56±4</td>
<td>-64±5</td>
<td>-69±4</td>
</tr>
<tr>
<td>10-mo, sham‡</td>
<td>-20±3</td>
<td>-29±3</td>
<td>-40±4</td>
<td>-42±4</td>
<td>-43±4</td>
</tr>
<tr>
<td>10-mo, lesioned*</td>
<td>-35±5</td>
<td>-45±5</td>
<td>-55±5</td>
<td>-56±6</td>
<td>-63±5†</td>
</tr>
<tr>
<td>F ratio</td>
<td>18.77</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Values are average±SEM from 11 2-month-old (2-mo) sham-operated, 10 2-month-old lesioned, 8 10-month-old (10-mo) sham-operated, and 10 10-month-old lesioned rats compared using a two-way analysis of variance with F ratios obtained for overall data. Data for nerve activity sampled for 2 seconds before and after each pressure point. All comparisons were made using Newman-Keuls multiple range test.

* p<0.05 comparing with data from age-matched, sham-operated group.
‡ p<0.05 comparing with corresponding average from age-matched, sham-operated group.

With data expressed as average±SEM, baselines for body weight, blood pressure, and heart rate were compared using an unpaired t test. All reflex responses to phenylephrine (Table 1) and responses to graded electrical stimulation of the afferent aortic nerve (Table 2) or the posterior hypothalamus (Table 3) were analyzed with a two-way analysis of variance. Reflex responses in heart rate and splanchnic nerve activity during intravenous infusions of phenylephrine were determined as changes occurring with every 10 mm Hg change in mean femoral pressure. For each infusion, five points for heart rate corresponding with mean pressure changes of 10, 20, 30, 40, and 50 mm Hg were thereby compiled. For electrical stimulation of the afferent aortic nerve (Table 2) or the posterior hypothalamus (Table 3), peak responses measured over 1–2 seconds were tabulated. Percent changes in sympathetic nerve frequency (Tables 1, 2, and 3) were ranked to verify that they were still distributed normally, and the two-way analysis of variance was used on both ranked and percent values. If both analyses gave similar results,
TABLE 2. Cardiovascular and Sympathetic Nerve Responses to Graded Electrical Stimulation of the Central Cut End of the Left Aortic Nerve in Rats Anesthetized With Urethane-Chloralose

<table>
<thead>
<tr>
<th>Rat groups</th>
<th>Aortic nerve stimulation (Hz)</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>Depressor response (mm Hg)</strong></td>
<td></td>
</tr>
<tr>
<td>2-mo, sham</td>
<td>-17±2</td>
</tr>
<tr>
<td>2-mo, lesioned*</td>
<td>-19±2</td>
</tr>
<tr>
<td>10-mo, sham†</td>
<td>-17±2</td>
</tr>
<tr>
<td>10-mo, lesioned*</td>
<td>-22±3</td>
</tr>
<tr>
<td>*F ratio=11.67, p&lt;0.0001</td>
<td></td>
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<tr>
<td><strong>Bradycardia (beats/min)</strong></td>
<td></td>
</tr>
<tr>
<td>2-mo, sham</td>
<td>-24±5</td>
</tr>
<tr>
<td>2-mo, lesioned*</td>
<td>-25±3</td>
</tr>
<tr>
<td>10-mo, sham</td>
<td>-18±3</td>
</tr>
<tr>
<td>10-mo, lesioned*</td>
<td>-20±4</td>
</tr>
<tr>
<td>*F ratio=6.59, p&lt;0.0005</td>
<td></td>
</tr>
<tr>
<td><strong>Sympathetic inhibition (% change)</strong></td>
<td></td>
</tr>
<tr>
<td>2-mo, sham</td>
<td>-24±4</td>
</tr>
<tr>
<td>2-mo, lesioned</td>
<td>-29±4</td>
</tr>
<tr>
<td>10-mo, sham†</td>
<td>-15±3</td>
</tr>
<tr>
<td>10-mo, lesioned*</td>
<td>-28±3</td>
</tr>
<tr>
<td>*F ratio=33.89, p&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

*Values are averages±SEM from 11 2-month-old (2-mo) sham-operated, 10 2-month-old lesioned, 8 10-month-old (10-mo) sham-operated, and 10 10-month-old lesioned rats compared using a two-way analysis of variance with F ratios obtained for overall data. Data for nerve activity sampled as peak responses for 1-2 seconds.

†p<0.05 comparing all data with that from age-matched, sham-operated group.

§p<0.05 comparing all data with that from 2-month-old, sham-operated group.

Results

**General Baselines**

Ten-month-old rats were heavier with slower heart rates and slightly higher blood pressures than 2-month-old rats. Averages obtained from 2- (n=35) and 10- (n=35) month-old rats anesthetized with urethane-chloralose were: 251±8 and 394±14 for body weight (in grams, p<0.0001); 309±5 and 283±6 for heart rate (in beats/min, p<0.001); and 114±2 for mean pressure.

TABLE 3. Effects on Mean Pressure and Splanchnic Nerve Activity of Stimulating the Posterior Hypothalamus Electrically in Rats Anesthetized With Urethane-Chloralose

<table>
<thead>
<tr>
<th>Rat groups</th>
<th>Current strength for stimulation (µA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50</td>
</tr>
<tr>
<td><strong>Pressor response (mm Hg)</strong></td>
<td></td>
</tr>
<tr>
<td>2-month-old</td>
<td>1±1</td>
</tr>
<tr>
<td>10-month-old*</td>
<td>2±1</td>
</tr>
<tr>
<td>*F ratio=25.31, p&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td><strong>Sympathetic excitation (% change)</strong></td>
<td></td>
</tr>
<tr>
<td>2-month-old</td>
<td>30±6</td>
</tr>
<tr>
<td>10-month-old*</td>
<td>35±6</td>
</tr>
<tr>
<td>*F ratio=31.92, p&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

*Values are averages±SEM from 14 2-month-old and 17 10-month-old rats compared using a two-way analysis of variance. Data for sympathetic excitation sampled as peak responses for 1-2 seconds.

†p<0.05 comparing all data with that from 2-month-old rats using Newman-Keuls multiple range test.

§p<0.05 as compared with corresponding average for 2-month-old rats using the Newman-Keuls multiple range test.
and 120±2 for mean femoral pressure (in mm Hg, p<0.05), respectively.

In rats subjected to sham operations or posterior hypothalamic lesions, age-related differences in body weight and heart rate persisted but those in blood pressure disappeared. Averages recorded 1 week later in 2-month-old sham-operated (n=11) and lesioned (n=10) rats under urethane-chloralose anesthesia were: 299±8 and 276±9 for body weight, 323±8 and 322±8 for heart rate, and 111±3 and 115±3 for mean pressure, respectively. Corresponding averages for 10-month-old sham-operated (n=8) and lesioned (n=10) rats were: 463±10 and 480±9 for body weight, 281±10 and 299±9 for heart rate, and 110±4 and 112±3 for mean pressure. Within each age group none of the differences between sham-operated and lesioned rats were significant (all p values >0.2).

Would Hypothalamic Lesions Alter Phenylephrine-Induced Reflex Responses Similarly Regardless of Age?

Inasmuch as baroreceptor reflex sensitivity also becomes impaired with age, it was considered possible that hypothalamic destruction would alter baroreceptor reflex sensitivity differently depending on age. To test this possibility, experiments were first done to determine whether removal of the posterior hypothalamus would affect reflex responses to phenylephrine similarly in both 2- and 10-month-old rats. Reflex heart rate and sympathetic nerve responses elicited as blood pressure was elevated by intravenous infusion of phenylephrine were recorded from rats with bilateral electrolytic lesions in the posterior hypothalamus and then compared with responses from age-matched, sham-operated controls.

As seen previously in intact rats, pressor responses elicited by intravenous infusions of phenylephrine were accompanied by reflex reductions in heart rate and splanchnic nerve firing, which in sham-operated rats were consistently weaker in the 10- than in the 2-month-old group (Table 1). Total doses of phenylephrine required to elevate mean pressure by 50 mm Hg were significantly higher in lesioned than in sham-operated rats for both age groups. Average doses (µg/100 g) in 2-month-old rats were 1.68±0.4 for sham-operated and 3.20±0.4 for lesioned groups (p<0.03); corresponding averages in 10-month-old rats were 1.41±0.4 and 2.54±0.4, respectively (p<0.01).

Magnitude of both reflex responses was larger in hypothalamic-lesioned than in sham-operated rats whether 2 (Figure 1) or 10 (Figure 2) months old. F ratios obtained from the two-way analysis of variance were highly significant, and comparisons using the Newman-Keuls test showed that in both age groups, rats with hypothalamic lesions had significantly larger reflex responses than corresponding sham-operated controls (Table 1). More importantly, although both reflex responses were consistently smaller in 10-month-old sham-operated than in 2-month-old sham-operated rats, corresponding age group differences among lesioned rats were not significant (Table 1). When the changes produced in the two age groups.
FIGURE 2. Representative tracings showing cardiovascular and sympathetic nerve responses to intravenously infused phenylephrine in two 10-month-old anesthetized rats. Left panel: Sham-operated rat. Right panel: Lesioned rat. From top to bottom tracings show: phasic pressure, mean pressure, heart rate, and splanchnic nerve activity. Arrows in each panel indicate start of 10-second stimulation period. Recorder speed 10 mm/sec.

were quantified separately (i.e., by subtracting responses in sham-operated rats from those in lesioned rats of the same group) and then compared with an analysis of variance, ensuing F ratios were 2.33 (p>0.1) for reflex bradycardia and 62.91 (p<0.0001) for reflex sympathetic inhibition. This indicates that baroreceptor reflex enhancement by the posterior hypothalamus for reflex sympathetic inhibition was significantly more pronounced in the 10- than in the 2-month-old rats. Because these results suggested

FIGURE 3. Representative tracings showing cardiovascular and sympathetic nerve responses to electrical stimulation of left aortic depressor nerve in two 2-month-old anesthetized rats. Left panel: Sham-operated rat. Right panel: Hypothalamic-lesioned rat. Stimulus strength indicated by numbers below each panel. From top to bottom tracings show: phasic pressure, mean pressure, heart rate, and splanchnic nerve activity. Recorder speed 5 mm/sec.
that destruction of the posterior hypothalamus had eliminated age-related changes in baroreceptor reflex sensitivity, it became critical to establish whether only reflex responses elicited with phenylephrine had been eliminated.

Are Responses to Afferent Aortic Nerve Stimulation Also Enhanced More in 10-Month-Old Rats With Hypothalamic Lesions?

To determine whether reflex responses to stimuli other than phenylephrine were likewise affected differently in the two age groups by hypothalamic lesions, additional experiments were done in the same rat groups to record blood pressure, heart rate, and splanchnic nerve activity during graded electrical stimulation of the left aortic depressor nerve. All three variables were consistently decreased during stimulation with 3-mA currents and within the range of current frequencies used (from 1 to 16 Hz) magnitude of each response gradually increased with stimulus frequency whether the rats were 2 (Figure 3) or 10 (Figure 4) months old. In sham-operated controls, decreases in mean blood pressure produced by stimulation with current frequencies of 1–4 Hz did not differ between age groups, but those produced by 8 and 16 Hz were smaller in 10-month-old rats (Table 2). With posterior hypothalamic lesions, magnitude of depressor responses in both age groups was significantly larger than that recorded from corresponding sham-operated controls. Associated decreases in heart rate did not differ between sham-operated groups (none of the group differences were significant) but were enhanced in rats with posterior hypothalamic lesions regardless of age (Table 2). By contrast, reductions in sympathetic nerve firing were considerably smaller in 10- than in 2-month-old
sham-operated rats, and posterior hypothalamic lesions selectively enhanced the sympathetic inhibition in 10- but not in 2-month-old rats (Table 2).

For comparing the magnitude of hypothalamic lesion effects in the two age groups, the changes in each group were quantified separately and then F ratios obtained with the analysis of variance were recorded. The F ratios were: 7.90, p<0.01 for depressor responses; 0.06, p>0.5 for bradycardia; and 4.67, p<0.05 for sympathetic nerve inhibition. These F ratios indicate that hypothalamic lesions enhanced depressor and sympathoinhibitory responses to afferent aortic nerve stimulation more strongly in 10- than in 2-month-old rats. Because sympathoinhibitory responses in 2-month-old rats were essentially unaltered, corresponding differences between lesioned groups were not significant (Table 2). Accordingly, these results show that by enhancing baroreceptor reflex sensitivity, differences existing between 2- and 10-month-old rats were abolished by electrolytic destruction of the posterior hypothalamus.

**Increased Hypothalamic Responsiveness and Lowered Thresholds in 10-Month-Old Intact Rats**

If age-related differences in reflex responsiveness can be eliminated by removal of the posterior hypothalamus, then responses to direct hypothalamic stimulation in intact rats should also differ with age. To test this reasoning, additional experiments were done to compare responses to graded electrical stimulation of the posterior hypothalamus with currents of the same frequency in 2- and 10-month-old rats. Regardless of age (i.e., whether the rats were 2 or 10 months old), electrical stimulation of the posterior hypothalamus always increased both blood pressure and splanchnic nerve firing (Figure 5). Attendant changes in heart rate were often biphasic and highly variable. On stimulation with increasing current strengths of constant frequency, pressor and sympathoexcitatory responses both increased progressively, and magnitude of each increase in response was consistently larger in 10- than in 2-month-old rats. Assessment of group differences with a two-way analysis of variance gave significant F ratios for both the pressor response and the attendant sympathetic excitation (expressed as percent increases in splanchnic nerve firing; Table 3).

To quantify group differences in levels of hypothalamic excitability a stimulus threshold, defined as the lowest current strength required to elicit a perceptible pressor or sympathetic nerve response, was measured. Threshold currents (µA) needed to elevate mean pressure by 2 mm Hg averaged 55±9 in 2-(n=14) and 28±8 in 10- (n=17) month-old rats (p<0.03); corresponding threshold currents for increasing sympathetic nerve activity by 20% were 30±11 and 8±6 (p<0.08), respectively. Because pressor and sympathetic responses to equal current strengths were larger (Table 3) and threshold currents required to elicit detectable pressor responses were lower in 10-month-old rats, these results collectively indicate that 10-month-old rats were more responsive than their 2-month-old counterparts to posterior hypothalamic stimulation.

**Postmortem Identification of Hypothalamic Electrode Placement and Lesion Sites**

Electrode tips and lesion sites were invariably located in the posterior hypothalamic nucleus. Adjacent structures included the mamillothalamic tract, zona incerta, fornix, dorsal premamillary nucleus, and anterovenital third ventricle (Figure 6).

**Discussion**

Even when blood pressure remains essentially unaltered, baroreceptor reflex suppression by the posterior hypothalamus evidently escalates with age. In line with this interpretation, we found that removal of the posterior hypothalamus by electrolytic lesioning equalized responses in 10- or 2-month-old rats to intravenously infused phenylephrine (Table 1) and to afferent aortic nerve stimulation (Table 2). By contrast, in age-matched, sham-operated rats (i.e., in whom the posterior hypothalamus was intact and presumably still able to inhibit baroreceptor reflex sensitivity) reflex responses to both phenylephrine and aortic nerve stimulation were consistently smaller in 10- than in 2-month-old rats. Furthermore, our subsequent finding of larger pressor and sympathoexcitatory responses to electrical stimulation of the posterior hypothalamus (Table 3) together with lower stimulus thresholds in 10-month-old rats, suggests that hypothalamic responsiveness had increased with age. However, it is also conceivable that pressor and sympathetic responses became enhanced because of diminished buffering by baroreceptor reflexes that were already impaired. Of these two possibilities, enhanced hypothalamic responsiveness seems more logical than baroreceptor reflex impairment because increases in sympathetic activity during hypothalamic stimulation usually preceded those in blood pressure (Figure 5). If so, then our results imply that by eliminating the baroreceptor reflex inhibition normally exerted by the posterior hypothalamus, its electrolytic destruction abolished age-related differences in baroreceptor reflex sensitivity.

Thresholds for electrical stimulation, determined by measuring current strengths required to elicit appreciable pressor responses, have previously been used in our laboratory to appraise the status of hypothalamic responsiveness under various experimental conditions. Elevated thresholds indicative of diminished responsiveness have been found after prolonged electrical stimulation, chronic exposure to shaker stress, and experimentally induced hypothyroidism. On the other hand, lowered thresholds indicative of increased responsiveness have been demonstrable only in old animals. On stimulating the posterior hypothalamus, Frolkis et al. obtained significantly lower pressor thresholds in 24-month-old white rats or 48-month-old rabbits than in corresponding younger controls. Although they studied...
FIGURE 6. Schematic diagram showing distribution of lesion sites in 10 rats from each group (o for 2-month-old and x for 10-month-old rats) with open arrows pointing to the posterior hypothalamus on both sides of the anteroventral third ventricle (shaded in black). Neighboring areas include the reuniens nucleus (RE), mamillothalamic tract (MT), zona incerta (ZI), fornix (FX), and dorsal premamillary nucleus (PMD). Anteroposterior coordinates indicated on upper left corner below the letter designating each panel. Millimeter scales for dorsoventral coordinates on the right and for lateral coordinates on the bottom.
different age groups, our findings generally agree in that we also found lower pressor thresholds in older rats. Unfortunately, because of the extensive surgical manipulation required in studies involving hypothalamic stimulation, in their experiments as well as in ours, the animals had to be anesthetized and possible artifacts due to anesthesia cannot be ruled out.

Like any other studies involving electrical stimulation or electrolytic destruction of discrete brain areas, the present studies must be interpreted with caution because neither procedure differentiates neuronal cell bodies from nerve fibers. Consequently, if fibers from other brain areas were stimulated or destroyed then the results obtained could reflect the influence of those brain areas rather than the posterior hypothalamus. Because the graded currents used here for stimulation could have spread to affect neural structures located away from the electrode sites, the larger responses elicited by stronger currents may in part reflect activation of adjacent brain areas. Alternatively, iron deposits formed from bipolar stainless steel electrodes used for stimulation or lesioning could differ between rat groups and thereby account for some of the ensuing functional differences. Also, because 10-month-old rats may have larger brains than 2-month-old ones (in unpublished experiments, we found brain weights of 1.89±0.05 g in 10-month-old rats as compared with 1.65±0.04 g in 2-month-old rats; p<0.003), the neuronal areas affected by either electrical stimulation or electrolytic destruction could have differed not only in location but also in size. Moreover, because neuronal pools within the brain overlap considerably, hypothalamic areas regulating body temperature, food intake, fluid balance, or sex behavior may have been stimulated or destroyed to alter humoral mechanisms that could indirectly influence the responses obtained. However, despite these difficulties in interpretation the results obtained here with either stimulation or lesioning of the posterior hypothalamus were remarkably consistent with each other in that, whereas hypothalamic stimulation revealed age-related differences in responsiveness and stimulus thresholds, the opposite procedure of hypothalamic destruction abolished age-related differences in baroreceptor reflex sensitivity.

An analogous relation between hypothalamic dysfunction and baroreceptor reflex impairment has also been described after chronic intracerebroventricular infusion of hypertonic salt. After 11 days of continuous intracerebroventricular infusion, depressor and sympathoinhibitory responses to electrical stimulation of the anterior hypothalamus were reduced as were reflex heart rate responses to phenylephrine or sodium nitroprusside. These findings were interpreted to mean that the infused hypertonic saline acted by depressing the anterior hypothalamus to reduce sympathoinhibition and impair baroreceptor reflexes. However, two major differences exist between those studies and ours. First, instead of being age-related, reflex responsiveness was altered by infusing saline intracerebroventricularly. And second, rather than arising from increased posterior hypothalamic activity, baroreceptor reflex impairment was caused by reduced anterior hypothalamic activity. The two hypothalamic areas affect reflex responses oppositely: the anterior hypothalamus facilitates reflex bradycardia and decreases sympathetic vasomotor tone, while the posterior hypothalamus suppresses reflex bradycardia and increases sympathetic vasomotor tone. Required communications are transmitted through neural pathways ascending from and descending to the nucleus tractus solitarii, which contain the first relay synapse of the baroreceptor reflex arc. Baroreceptor reflex regulation by the hypothalamus then normally depends on a balance of opposing actions exerted on the reflex arc such that impairment could result from either decreasing anterior hypothalamic facilitation or increasing posterior hypothalamic suppression. Thus, baroreceptor reflex impairment, which can be commonly caused either by intracerebroventricular-infused saline or by increasing age, would be due to different mechanisms involving either decreased anterior hypothalamic or increased posterior hypothalamic activity.

Exactly how aging affects the hypothalamus to alter baroreceptor reflex regulation remains conjectural. Baroreceptor reflex impairment in old rats probably does not depend on either hypertension (i.e., baselines for mean pressure in our sham-operated groups were 111±3 in 2-month-old and 110±4 mm Hg in 10-month-old rats) or atherosclerosis. Although the incidence of atherosclerosis in Sprague-Dawley rats is unknown, the Fischer 344 rats usually used in aging research do not develop atherosclerosis. Moreover, because we compared young mature (2-month-old) with middle-aged (10-month-old) rats, the applicability of our present findings to older age groups is uncertain. Nevertheless, according to the neuroendocrine theories on aging and age-related changes are caused by neuronal and hormonal deficiencies resulting from diminished function of the hypothalamic-pituitary axis. In line with such theories, extensive structural changes have been found in the hypothalamus with age, but they have yet to be linked to functional defects that could influence baroreceptor reflex or cardiovascular regulation.

In summary, we have shown in rats anesthetized with urethane-chloralose that removal of the posterior hypothalamus alters baroreceptor reflex sensitivity differently depending on age. Reflex bradycardia and sympathoinhibition elicited during pressor responses to intravenously infused phenylephrine were stronger in rats with bilateral hypothalamic lesions than in age-matched sham-operated controls, as were responses to afferent aortic nerve stimulation. In 10-month-old rats, hypothalamic lesions not only enhanced reflex responses but also restored sensitivity to levels comparable with those in 2-month-old rats. Because this indicated that age-related differ-
ences in baroreceptor reflex sensitivity had been abolished by electrolyte destruction of the posterior hypothalamus, additional experiments were performed to determine whether age-related differences in responsiveness to direct hypothalamic stimulation could also be detected. On electrical stimulation of the posterior hypothalamus, pressor and sympathoexcitatory responses were larger, but stimulus thresholds were lower in 10- than in 2-month-old rats. These results are in accord with the interpretation that aging escalates the baroreceptor reflex suppression normally exerted by the posterior hypothalamus.

References


KEY WORDS • aging • baroreceptor reflex • blood pressure • heart rate • hypothalamus • sympathetic nerve activity
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