Central control of cardiac baroreflex responses during peripheral hyperosmolality

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Bealer, Steven L. Central control of cardiac baroreflex responses during peripheral hyperosmolality. Am J Physiol Regulatory Integrative Comp Physiol 278: R1157–R1163, 2000.—Acute increases in peripheral osmolality evoke a pressor response and baroreflex-mediated bradycardia. These experiments were designed to determine if the fall in heart rate during peripheral sodium loading is 1) equivalent to bradycardia accompanying phenylephrine (PE) infusion, 2) mediated by the parasympathetic (PSNS) or sympathetic (SNS) nervous system, and 3) controlled by the median preoptic nucleus (MnPO). Male rats received an intravenous infusion of isotonic saline, hypertonic saline (2.5 M NaCl), or PE for 30 min. Blood pressure increased equivalently in the hypertonic NaCl and PE groups. However, heart rate fell more in animals infused with PE. Furthermore, pretreatment with methylatropine to block the PSNS had no effect on bradycardia, whereas blocking SNS influences on cardiac function significantly attenuated the fall in heart rate during peripheral hyperosmolality. Finally, kainic acid administration in the MnPO before testing increased bradycardia observed during hypertonic saline loading. Taken together, these data suggest that acute peripheral hyperosmolality acts at the MnPO to reduce cardiac SNS withdrawal during the pressor response that reduces the associated baroreflex bradycardia.

Increased plasma osmolality has several effects that may increase blood pressure, including activation of the sympathetic nervous system (SNS) (1, 9, 13), increased vasopressin release (1, 9), and expanded extracellular fluid volume. Failure to adequately compensate for hyperosmotic activation of these pressor systems may lead to transient or sustained hypertension. Perivascular infusion of hypertonic NaCl, which increases blood pressure, produces a fall in heart rate (1, 9, 20) that is mediated by stimulation of arterial baroreceptors (20). Because baroreflex-induced bradycardia is a primary mechanism of blood pressure maintenance, it is important to understand the functional effect of hyperosmolality on cardiac baroreflex responses.

However, the nature of the baroreflex-induced bradycardia during peripheral hyperosmolality is not completely characterized. For example, the relationship between blood pressure and heart rate during the hypertonic saline (HTS)-induced pressor response is not quantified. Several circulating pressor agents, such as ANG II (2, 24) and vasopressin (8, 33), evoke baroreceptor-induced decreases in heart rate and/or SNS activity, which are modified by an action of the circulating substance on the central nervous system (CNS). Because the cardiovascular effects of peripheral hyperosmolality are evoked by central mechanisms that could modify baroreceptor-mediated responses, one objective of these experiments was to compare bradycardia evoked by intravenous infusion of hypertonic NaCl to that produced by equipressor doses of phenylephrine (PE).

Furthermore, the precise roles of the SNS and parasympathetic (PSNS) components of the autonomic nervous system in regulating the baroreflex-induced cardiac responses during peripheral sodium loading are not elucidated. Bradycardia during short-term (<5 min) pressor responses is mediated primarily by activation of the PSNS, whereas the decrease in heart rate observed during sustained increases in blood pressure is due primarily to withdrawal of cardiac SNS tone (7, 40, 41). Because intravenous HTS infusion stimulates the SNS (9, 13), PSNS activation may contribute to the associated fall in heart rate. Therefore, a second objective of these studies was to determine the role of the PSNS in bradycardia during peripheral saline loading. Finally, the central sites that regulate changes in baroreflex sensitivity induced by hyperosmolality are not determined. However, a previous study by Bealer (3) demonstrated the periventricular tissue surrounding the anteroventral portion of the third cerebral ventricle (AV3V) contains neurons critical for the cardiovascular effects of HTS infusion. One site within the AV3V area that may contribute to the cardiovascular effects and baroreflex responses during hypertonic sodium loading is the median preoptic nucleus (MnPO). This brain site is responsive to osmolality (28, 37) and has been directly implicated in regulation of cardiac baroreflex sensitivity (16, 26). Therefore, a third objective of these experiments was to determine the role of the MnPO in bradycardia associated with pressor responses produced during increased peripheral osmolality.

METHODS

Animals. Male Sprague-Dawley rats were purchased from Harlan and housed in hanging wire cages in a room on a 12:12-h light-dark cycle. Animals had ad libitum access to food and water and weighed between 250 and 310 g at the time of testing.
Surgery. On the day before the experiment, rats were anesthetized with methohexital sodium (Brevital; 60 mg/kg ip). The animals were implanted with heparin-saline (50 U/ml)-filled polyethylene catheters (40 mm PE-10 cemented in PE-50) in one femoral artery and one femoral vein, and they were then returned to their home cage to recover overnight.

Some animals received chemical lesions of the MnPO 7 to 10 days before testing. These rats were anesthetized with methohexital sodium (60 mg/kg ip) and placed in a stereotaxic instrument. The scalp was incised and the skull leveled between lambda and bregma, and a 2-mm hole was centered on bregma and drilled through the skull. A 30-gauge stainless steel injector was lowered into the ventral portion of the MnPO (0.2–0.3 mm anterior to bregma, 0.0 mm lateral to the midline, and 6.3–6.5 mm ventral to the dura). The injector was connected to a remote microsyringe that was back filled with kainic acid (Sigma, 4.0 mg/ml) or with artificial cerebrospinal fluid. Kainic acid or vehicle (0.25 µl) was administered into the MnPO over 15 s, and the injector remained in place for an additional 45 s to allow diffusion of the solution away from the injector tip. Administration of kainic acid with the use of these procedures produces a cell body lesion localized within the MnPO (18). Rats were returned to their home cages and were undisturbed until prepared for testing as described above.

Protocol: experiment 1. The conscious, unrestrained rats were placed in a plastic cage, and the arterial catheter was connected to a pressure transducer and MacLab data-acquisition system. Blood pressure was continuously measured, and heart rate was calculated by an internal ratemeter in the data-acquisition system. The venous catheter was connected to a remote syringe in a syringe pump with polyethylene tubing. The animals were given 45–60 min to equilibrate.

After equilibration, rats received a 30-min infusion of isotonic saline (0.15 M; 0.1 ml·kg⁻¹·min⁻¹), hypertonc NaCl (2.5 M; 0.1 ml·kg⁻¹·min⁻¹), or PE (1–75 µg·kg⁻¹·min⁻¹). The rate of PE administration was continuously adjusted throughout the infusion period so that the increase in blood pressure was approximately equivalent to the pressor response observed during infusion of hypertonic NaCl.

Protocol: experiment 2. Other animals were administered similar infusions of HTS. However, 10 min before the initiation of the infusion period, these rats were given a bolus intravenous injection of either methylatropine (Sigma Chemical; 1 mg/kg) or atenolol (Sigma Chemical; 1 mg/kg). These treatments are shown to abolish PSNS and SNS influences on cardiac function, respectively (22, 38).

An attenuated bradycardia during HTS infusion after atenolol treatment may result from the significant decrease in resting heart rate that follows β-adrenergic blockade. To test this possibility, cardiac responses to bolus injections of PE were examined in vehicle-treated and in atenolol-treated animals. In these groups, rats were given a single bolus injection of PE to raise blood pressure 35–45 mmHg within 30–45 s. 10 min after atenolol (1 mg/kg) or saline administration. The maximum changes in blood pressure and heart rate after this procedure were recorded.

Protocol: experiment 3. Finally, rats with kainic acid lesions in the MnPO and animals that underwent control surgical procedures received a 30-min infusion of HTS as described above. After the experiment, these rats were anesthetized with pentobarbital sodium, and the brains were transcardially perfused with saline followed by Formalin-saline solution. Brains were stored in sucrose Formalin, subsequently frozen, sectioned through the region of the MnPO, and mounted on slides. After staining with cresyl violet, the position and extent of kainic acid damage was evaluated using the light microscope.

Data collection and analysis. Control blood pressure (mmHg) and heart rate (beats/min) were taken as the average values measured over a 60- to 90-s period 5 min before the initiation of the infusion period. The infusion was not started on any animal that did not have stable blood pressure and heart rate. During the infusion period, 1-min averages of blood pressure and heart rate values were obtained at 5-min intervals. The mean control values were subtracted from each of these averages to obtain the differences in blood pressure and heart rate during the infusion period (see Figs. 1, 2, 5).

Data in Fig. 3 represent the maximum changes in blood pressure and heart rate obtained after intravenous administration of PE.

Data are reported as means ± SE. Data were compared with the use of a two-factor analysis of variance with repeated measures. Differences between individual means were evaluated with a Newman-Keuls test.

RESULTS

Experiment 1. Before the infusion period, control blood pressure and heart rates were not different between animals subsequently infused with 0.15 M NaCl (125 ± 5 mmHg; 382 ± 12 beats/min), 2.5 M NaCl (126 ± 4 mmHg; 388 ± 17 beats/min), or PE (124 ± 4 mmHg; 380 ± 12 beats/min). Furthermore, infusion of 0.15 M NaCl or PE did not alter plasma osmolality, whereas administration of 2.5 M NaCl increased plasma osmolality 18 ± 3 mosmol/kgH₂O. Figure 1 summarizes the changes in blood pressure and heart rate produced by the 30-min infusion of 0.15 M NaCl, 2.5 M NaCl, or PE. Infusion of isotonic saline did not change either blood pressure or heart rate. However, administration of HTS or PE resulted in a significant pressor response and bradycardia. Although the increase in blood pressure was similar, bradycardia during the infusion period was significantly smaller at 25 and 30 min in animals receiving HTS infusion compared with animals given PE.

Experiment 2. Control blood pressures and heart rates were similar before injections of methylatropine (124 ± 5 mmHg; 385 ± 10 beats/min) or atenolol (126 ± 4 mmHg; 378 ± 8 beats/min). In addition, there were no effects of either pharmacological agent on blood pressure. However, methylatropine significantly increased heart rate (453 ± 11 beats/min), whereas atenolol produced significant bradycardia (338 ± 8 beats/min).

Infusion of HTS produced equivalent increases in plasma osmolality in both groups of animals (methylatropine, 16 ± 3 mosmol/kgH₂O; atenolol, 18 ± 3 mosmol/kgH₂O). Changes in blood pressure and heart rate during HTS infusion in nontreated rats and in animals given intravenous methylatropine or atenolol are illustrated in Fig. 2. Prior treatment with either the muscarinic or the β-adrenergic antagonist did not alter the pressor response to HTS infusion. Furthermore, animals given intravenous methylatropine before the infusion period demonstrated a fall in heart rate similar to that observed in nontreated animals receiving HTS. However, bradycardia in rats receiving atenolol before HTS infusion was significantly attenuated compared with nontreated, HTS-infused animals. These data...
demonstrate that the fall in heart rate mediated by baroreceptor stimulation during HTS infusion is due predominantly to withdrawal of cardiac SNS tone. It is possible that the decrease in heart rate after atenolol treatment could reduce the range of bradycardia during subsequent baroreceptor stimulation. To test this possibility, the effects of β-adrenergic blockade on the heart rate response induced by a bolus injection of PE were examined. Again, there were no differences in blood pressure or heart rate in vehicle-treated (123 ± 6 mmHg; 374 ± 6 beats/min) and atenolol-treated (127 ± 5 mmHg; 382 ± 6 beats/min) animals before drug administration. In addition, intravenous injection of vehicle did not alter blood pressure (123 ± 4 mmHg) or heart rate (374 ± 11 beats/min), and atenolol did not change blood pressure (130 ± 4 mmHg). However, atenolol again resulted in a significant fall in heart rate (345 ± 10 beats/min). The maximum change in blood pressure and heart rate after a bolus intravenous injection of PE in vehicle- and atenolol-treated animals is shown in Fig. 3. Although control heart rate was significantly lower in atenolol-treated animals compared with vehicle-treated rats, the fall in heart rate during the PE-induced pressor response was similar. These data demonstrate that the attenuated bradycardia during HTS infusion in atenolol-treated animals was not due to the reduced basal heart rate after β-adrenergic blockade.

Experiment 3. Figure 4 shows photomicrographs of coronal brain sections taken through the level of the MnPO from animals receiving injections of either vehicle (Cont; Fig. 4, left) or kainic acid (MnPO X; Fig. 4, right). In animals receiving kainic acid injections, lesion damage was well confined to the MnPO, within 150 µm of the midline. As previously described, in similarly treated animals, the lesion area contained dark staining and an accumulation of pyknotic cells and debris (18).

Control blood pressures from MnPO X animals (127 ± 4 mmHg) and Cont animals (119 ± 5 mmHg) were both similar to control blood pressure in animals infused with PE (126 ± 4 mmHg; data from experiment 1). In addition, control heart rates were not different between Cont (375 ± 12 beats/min) and PE-infused (380 ± 12 beats/min; data from experiment 1) groups. However, cell body ablation in the MnPO produced significant tachycardia (412 ± 7 beats/min).

During HTS infusion, plasma osmolality increased equivalently in Cont (19 ± 3 mosmol/kgH₂O) and MnPO X (16 ± 3 mosmol/kgH₂O) animals. Figure 5 summarizes the changes in heart rate and blood pressure observed during HTS infusion in animals with lesions in the MnPO (HTS-MnPO X), in control-operated animals (HTS-Cont), and in rats infused with PE (data reproduced from Fig. 1). Blood pressure increased equivalently in all groups during the infusion period. However, bradycardia induced by hypertonicity...
in animals with kainic acid lesions of the MnPO was significantly greater than the fall in heart rate observed in control-operated animals. Indeed, bradycardia in animals with lesions in the MnPO during HTS infusion was similar to that observed in neurologically intact animals receiving PE infusions. These findings demonstrate that neurons in the MnPO make a significant contribution to inhibition of cardiac responses during HTS infusion.

DISCUSSION

The results of these experiments demonstrate that the decrease in heart rate associated with the pressor response resulting from an intravenous infusion of hypertonic NaCl is reduced compared with bradycardia evoked by an intravenous infusion of equipressor doses of PE. Furthermore, the reflex-induced bradycardia during a 30-min HTS infusion is due predominantly to withdrawal of cardiac SNS tone. Finally, destruction of nerve cell bodies in the MnPO enhances bradycardia evoked by hyperosmolarity, making it equivalent to that observed during PE.

Several previous studies (6, 30, 39) reported that administration of hypertonic solutions into the CNS reduces baroreflex sensitivity. For example, a bolus injection of 1.0 M NaCl into the lateral cerebral ventricle decreases the gain and range of the cardiac response during acute pressor responses when tested 1 h after administration (39). Furthermore, chronic infusion of hypertonic solutions into the third cerebral ventricle increases blood pressure without a concomitant decrease in SNS activity (30) and reduces baroreflex-induced cardiac responses to acute increases in blood pressure (6). The findings from the present studies extend these earlier results by demonstrating an attenuated baroreflex-induced fall in heart rate during peripheral HTS infusion.

Other circulating pressor substances modify baroreflex-induced changes in heart rate and/or SNS activity through an action at sites in the CNS. For example, bradycardia induced by the pressor effects of intravenous angiotensin II is attenuated compared with that observed during PE infusion due to an action of the peptide at the area postrema (24, 27) and/or in the AV3V region (2). Circulating vasopressin also acts at the area postrema to enhance baroreflex-mediated responses relative to PE (8, 33). The present experiments demonstrate that peripheral hyperosmolality also alters CNS processing of baroreceptor information to diminish cardiac responses.

Withdrawal of SNS activity is the primary component of baroreflex-induced cardiac responses during sustained increases in blood pressure to PE (7, 40, 41). However, an intravenous infusion of HTS increases SNS activity (13). Therefore, the relative contributions of the SNS and PSNS to cardiac responses during this pressor treatment may be altered. The present experiments directly tested this possibility. These studies found that a blockade of SNS cardiac responses significantly attenuated bradycardia during peripheral hyperosmolality, whereas a PSNS blockade had no effect. Therefore, as reported for other pressor agents, the predominant mechanism of bradycardia during the sustained pressor response evoked by intravenous HTS infusion is withdrawal of cardiac SNS tone.

Results from the present studies found that chemical lesions of the MnPO increased bradycardia during HTS infusion, suggesting that hyperosmolality and/or sodium is acting at this brain site to attenuate pressor-induced bradycardia during HTS infusion. This interpretation is consistent with a number of studies demonstrating that the MnPO contains osmosensitive...
It is unlikely that the volume expansion associated with infusion of HTS could account for the observed effects on heart rate as volume expansion does not change either blood pressure or heart rate. In previous experiments, infusion of isotonic saline at a rate adjusted to administer sodium at the same rate as the 2.5 M NaCl infusion used in these studies did not significantly change blood pressure or heart rate when infused for 30 (3) or 60 (4) min. These findings are in agreement with earlier demonstrations that volume expansion does not contribute to the pressor response evoked by infusion of HTS in anephric rats (13). Furthermore, it is unlikely that vasopressin release can account for attenuated bradycardia associated with HTS infusion, because both exogenous and endogenous vasopressin enhance, not reduce, the baroreflex-induced fall in heart rate during pressor responses (12, 33).

An additional finding of these experiments was that kainic acid lesions placed in the MnPO result in significant tachycardia 7 to 10 days after placement of the lesion. This result supports other studies suggesting the AV3V region in general, and perhaps the MnPO specifically, contribute to autonomic nervous system control of heart rate. For example, electrolytic ablation of the AV3V periventricular tissue produces a transient increase in heart rate that returns to normal within 14 days (21). In addition, local administration of the specific α1-adrenergic antagonist, prazosin, to the AV3V region results in acute tachycardia in chloralose-anesthetized rats (5). Finally, electrolytic lesions localized to the MnPO transiently increase heart rate (26).

Although this brain region appears to contribute to maintenance of normal resting heart rate in neurologically intact rats, the precise roles of the PSNS and SNS in posttreatment tachycardia and the mechanism of subsequent recovery of normal heart rate have not been systematically investigated.

Although the increase in basal heart rate after chemical lesions in the MnPO observed in these studies could result from decreased PSNS tone or increased SNS activity, it is unlikely that the attenuated bradycardia observed during the pressor response to intravenous HTS infusion is due to diminished PSNS responsiveness. This fall in heart rate is predominantly due to withdrawal of PSNS activity, because it was almost eliminated by β-adrenergic blockade, but is unaffected by peripheral administration of a muscarinic antagonist. Furthermore, lesions in the MnPO increased bradycardia during intravenous HTS infusion, which is inconsistent with diminished PSNS tone in any experimental condition. Taken together, these data suggest that the effects of MnPO ablation on cardiac responses to peripheral hyperosmolality are due to enhanced SNS withdrawal.

Although it is unlikely that decreased PSNS drive after MnPO lesions contributed to diminished heart rate responses during 30-min HTS infusions in the current experiments, there is evidence that this brain region can also affect PSNS control of cardiac function. Manning et al. (26) demonstrated that electrolytic

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Fig. 5. Changes in blood pressure and heart rate during 30-min infusion of hypertonic NaCl in animals receiving control (Cont) injections in MnPO (HTS-Cont; n = 8) and animals receiving kainic acid injections in the MnPO (HTS-MnPO X; n = 6). Also shown are changes in cardiovascular variables in animals infused with PE (data reproduced from Fig. 1; n = 6). *P < 0.05; **P < 0.01 HTS-Cont compared with HTS-MnPO X.
lesions of the MnPO diminish bradycardia to bolus injections of PE that is mediated predominantly by PSNS activation (7, 40, 41). In conjunction with results from the present studies, these data suggest that the MnPO is an important CNS site that can regulate cardiac function through either the SNS or PSNS components of the autonomic nervous system under different experimental conditions.

It is possible that the greater bradycardia in animals with MnPO lesions may have resulted from the higher resting heart rate in this group of animals. However, animals treated with methylatropine also had significantly increased basal heart rate, but exhibited bradycardia comparable to untreated rats during HTS infusion. In addition, in an earlier experiment (5), prazosin administration in the preoptic recess similarly increased heart rate, but did not alter baroreflex-induced bradycardia. Therefore, it appears unlikely that tachycardia per se can account for the enhanced fall in heart rate observed during HTS infusion in animals with neurotoxic lesions in the MnPO.

Bradycardia evoked by infusion of HTS was reduced by ~48% compared with the fall in heart rate observed during infusion of PE (~38 ± 10 beats/min; ~73 ± 9 beats/min, respectively). This attenuation of the cardiac response is equivalent to that reported in spontaneously hypertensive rats (15) and during the development of one-kidney, one-clip hypertension (31), which have been proposed to contribute to the development of hypertension in these experimental models. Furthermore, this difference is similar to the reduction of the cardiac response after consumption of a high-sodium diet (14) and to the enhancement of baroreflex-induced bradycardia evoked by vasopressin (33), both of which are considered to make significant contributions to blood pressure regulation. Therefore, it is likely that the degree of attenuated bradycardia observed during hyperosmolality significantly alters the responses of other systems activated or inhibited by baroreceptor stimulation to maintain blood pressure stability.

In the present experiments, atenolol blocked the baroreflex-mediated decrease in heart rate, but the pressor response was unaffected. This suggests that cardiac and neural components of the baroreflex may be independently regulated. It is possible that during the diminished pressor-induced fall in heart rate, neural sympathetic withdrawal to the vasculature is increased, thus preventing an enhanced pressor response. There is other evidence suggesting independent control of cardiac and neural baroreflex responses. For example, altering dietary sodium produces differential effects on heart rate and sympathetic nerve responses to changes in blood pressure (15). In addition, the MnPO selectively enhances the cardiac response to increases in blood pressure induced by vasopressin and has no effect on neural responses (32).

Electrolytic ablation of the AV3V region prevents the pressor effects of hyperosmotic stimuli (17). However, the specific role of neurons in the MnPO in mediating the increase in blood pressure to osmotic stimulation is not addressed. Some studies suggest that the MnPO is not involved in pressor responses associated with the AV3V region. For example, an early experiment evaluating the effects of electrical microstimulation of structures within the AV3V area found that stimulation of the MnPO decreased blood pressure (23). In addition, kainic acid lesions localized to the MnPO do not reduce the pressor response to ANG II (18) that is abolished by electrolytic ablation of the entire AV3V area (17). Therefore, it is not entirely unexpected that neurotoxic lesions confined to the MnPO failed to alter the increase in blood pressure induced by HTS infusion.

In summary, these experiments demonstrate that the baroreflex-mediated bradycardia observed during intravenous infusion of HTS is reduced compared with the fall in heart rate that accompanies equipressor doses of PE. Furthermore, attenuated bradycardia during HTS infusion appears to be due to maintained SNS drive. Finally, ablation of MnPO nerve cell bodies increases the baroreflex-induced fall in heart rate during peripheral hypertonicity. Taken together, these data are consistent with the proposal that peripheral hyperosmolality acts at or through the MnPO to reduce cardiac SNS withdrawal during the pressor response that then attenuates the baroreflex-induced bradycardia.

Perspectives

A number of studies (6, 30, 39) demonstrated that changes in body sodium status and/or sodium sensitivity can have profound effects on blood pressure, heart rate, and baroreflex responses. For example, central sodium loading increases blood pressure and decreases baroreflex sensitivity. Furthermore, chronic intake of a high-sodium diet also diminishes cardiac baroreflex responsiveness (14, 15). Finally, sodium-sensitive hypertension is accompanied by decreased baroreflex sensitivity (11, 41) that may contribute to the increase in blood pressure. Failure of baroreflex-induced bradycardia to compensate for the pressor effects of acute or chronic sodium loading may be a contributing factor to increased blood pressure variability and to transient and/or sustained increases in blood pressure associated with alterations in plasma sodium or increased sodium ingestion.

Results from the present study suggest that intravenous sodium and/or osmolality can act either directly, or indirectly through synaptic inputs, at the MnPO to modify SNS output and decrease cardiac baroreflex sensitivity. This CNS circuit may be activated and contribute to reduced baroreflex responses by modifying SNS tone during central sodium administration, increased sodium diet, and/or sodium-sensitive hypertension.

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