Cardiovascular effects of hypocretin-1 in nucleus of the solitary tract

Cleusa V. R. de Oliveira, M. Patricia Rosas-Arellano, L. Pastor Solano-Flores, and John Ciriello
Department of Physiology and Pharmacology, Faculty of Medicine and Dentistry, Health Sciences Center, University of Western Ontario, London, Ontario, Canada N6A 5C1
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Hypocretin (Hcrt) neuropeptides have been recently shown to be almost exclusively expressed within neurons of the lateral and perifornical hypothalamic areas (30, 33, 35, 47, 49). These peptides, Hcrt-1, a 33 amino-acid peptide with an NH2-terminal pyroglutamyl residue, and Hcrt-2, a 28 amino acid peptide with a COOH-terminal amide (35), are derived from the same long preproorexin molecule by proteolytic cleavage (35). Hcrt-2 has been reported to have 46% similarity with Hcrt-1 (35), and both peptides bind and activate G protein-coupled receptors (11, 35). Although both Hcrt-1 and -2 have been shown to have similar physiological effects when administered centrally, Hcrt-1 appears to exert a more potent effect on most of these physiological variables (10, 12, 33, 42, 47). Hcrt-1 injections into the brain have been shown to increase feeding and drinking behaviors; produce sleep, wakefulness, and arousal disturbances; produce analgesia; activate the hypothalamic-pituitary axis; elicit gastric acid secretion; and alter temperature regulating mechanisms (3, 14, 19, 21, 23, 25, 42, 44, 45, 48). Hcrt-1 neurons have been shown to contribute to an extensive innervation of forebrain, brain stem, and spinal cord structures (10, 33, 42, 46, 47), and receptors to Hcrt-1 have been demonstrated throughout the neuraxis (28).

Recently, it has been suggested that Hcrt-1 may exert an effect on neuronal circuits that control the cardiovascular system (10, 36). Injections of Hcrt-1 into the lateral cerebral ventricles has been shown to elicit increases in renal sympathetic activity and catecholamine release, and a long-lasting increase in arterial pressure (29, 39), which may be due to increased release of vasopressin into the circulation (29). In addition, direct injections of Hcrt-1 into the paraventricular nucleus of the hypothalamus have also been shown to elicit a long-lasting increase in heart rate (HR) (37). These latter findings are consistent with the observation that Hcrt-1 increases the discharge rate of paraventricular nucleus neurons (38). Intracisternal injections of Hcrt-1 have also been reported to elicit a dose-dependent increase in arterial pressure and HR (4). These effects have been suggested to be mediated by the activation of sympathetic premotor neurons in the rostral ventrolateral medulla (5, 14). Injections of Hcrt-1 into the rostral ventrolateral medulla elicited an increase in arterial pressure and HR (4). Finally, it has been shown that intrathecal injections of Hcrt-1 elicit increases in arterial pressure and HR, effects
suggested to be mediated by activation of sympathetic preganglionic neurons in the intermediolateral nucleus of the thoracolumbar cord (2).

In addition to the finding of Hcrt-1-labeled fibers in cardiovascular regions of the ventrolateral medulla and spinal cord, it has been reported that the nucleus of the solitary tract (NTS) receives a direct projection from Hcrt-1-containing neurons within the lateral hypothalamus (20). Furthermore, intracerebroventricular injections of Hcrt-1 have been shown to induce c-fos expression in the NTS (10). Because the NTS is the primary site of termination of cardiovascular afferent fibers (7), this observation suggests the possibility that Hcrt-1 may exert an effect on NTS circuits controlling the circulation. A recent report (40) has shown that large (500 nl) injections into the commissural nucleus of the NTS complex elicit increases in arterial pressure and HR.

Two series of experiments were done in this study to determine the effect of Hcrt-1 into regions of the NTS complex that receive baroreceptor afferents (7) on arterial pressure and HR. In the first series, because a detailed mapping of Hcrt-1-like immunoreactivity (Ir) throughout the NTS complex, especially within the cardiovascular responsive regions of NTS, is not available, the distribution of Hcrt-1-like Ir was mapped in the rat. In second series, the effect of discrete microinjection of Hcrt-1 into the NTS complex on arterial pressure, HR, and reflex bradycardia to activation of the baroreceptor reflex was investigated in the anesthetized rat. In addition, studies were done to investigate the components of the autonomic nervous system mediating these cardiovascular effects.

METHODS AND MATERIALS

General procedure. Experiments were done in adult male Wistar rats (250–300 g; Charles River Canada, St. Constant, Canada). All animals were housed under controlled conditions with a 12:12-h light/dark cycle. Food and water were available to all animals ad libitum. All experimental procedures were done in accordance with the guidelines on the use and care of laboratory animals as set by the Canadian Council on Animal Care and approved by the Animal Care Committee at The University of Western Ontario.

Immunohistochemistry. Four animals under pentobarbital sodium anesthesia (65 mg/kg ip; MTC Pharmaceuticals, Cambridge, ON, Canada) were perfused transcardially with 500 ml of 0.9% physiological saline, followed by 500 ml of Zamboni’s fixative containing 2% paraformaldehyde in 0.1 M phosphate buffer at pH 7.2–7.4 and 15% saturated picric acid at 4°C. Brains were removed and stored in a 10% sucrose-PBS solution overnight. Frozen, serial, transverse sections of the brain stem at 40 μm were cut in a cryostat (−17°C; model 5030; Bright Instrument, Huntington, UK). For each animal, one in every two sections of the brain stem were processed immunohistochemically as previously described (6) for Hcrt-1 Ir. Brain stem sections were placed in normal goat serum (Vector Laboratories, Burlingame, CA) diluted 1:50 with PBS containing 0.3% Triton X-100 for 30 min. Sections were then rinsed in PBS and placed in primary antiserum to Hcrt-1 (affinity-purified rabbit polyclonal anti-orexin-A; model OXA11-A; Alpha Diagnostic International, San Antonio, TX) (11, 35) diluted 1:2,000 in PBS/0.3% Triton X-100 at 4°C. After 72 h, the sections were rinsed in PBS and placed for 30 min in goat biotinylated anti-rabbit IgG (Vector Laboratories) diluted 1:500 in PBS/0.3% Triton X-100. After being rinsed in PBS, sections were placed in a solution of methanol and hydrogen peroxide (29:1) for 30 min. Sections were then rinsed in PBS and placed in an avidin-biotin complex reagent ( Vectastain ABC Elite Kit) in PBS/0.3% Triton X-100 for 75 min and then washed again in acetate buffer at pH 5.5. Peroxidase contained in the ABC reagent was visualized by placing sections in a solution of 0.006% hydrogen peroxide and 0.02% 3,3’-diaminobenzidine tetrahydrochloride (DAB; Sigma, St. Louis, MO) in PBS for 20 min, or in 0.05% DAB, 0.05% hydrogen peroxide, and 0.01% nickel ammonium sulfate in acetate buffer for 15–20 min. After the tissue was rinsed in PBS, sections were mounted onto gelatinized glass slides, dried, and coverslipped. Brain stem sections adjacent to those processed for Hcrt-1 Ir were stained with either neutral red or thionine for the identification of cytoarchitectonic boundaries. Analysis was done by using bright- and dark-field microscopy. Location of Hcrt-1-like Ir-labeled fibers was mapped onto camera lucida projection drawings of the dorsomedial medulla for each experimental case.

Controls for Hcrt-1-like Ir included placing brain stem sections in primary Hcrt-1 antiserum that had been preabsorbed with an excess of Hcrt-1 peptide (catalog no. OXAI1-P; Alpha Diagnostic) or sections in which the reaction of the tissue with the primary antisera was omitted (6). Under these conditions, no Hcrt-1-like Ir was demonstrated in the brain stem sections.

Microinjection into NTS. On the day of the experiments, animals were anesthetized with a-chloralose (80 mg/kg iv initially, and supplemented by additional doses of 10–20 mg/kg given every 1–2 h; n = 10) after induction with equithesin (0.3 ml/100 g ip). In addition, to determine whether the type of anesthesia used qualitatively or quantitatively altered the cardiovascular responses, experiments were also done in animals anesthetized with urethane (1.5 g/kg ip; n = 10). The trachea was cannulated and the animals were artificially ventilated by the use of a small rodent ventilator (model 683; Harvard Apparatus) with a mixture of room air and 95% of O2. Body temperature was maintained at 36–37°C by a heating pad (model K-20-C; American Hospital Supply, Cincinnati, OH). Polyethylene catheters-50 (Clay Adams, Parsippany, NJ) were inserted into the femoral artery and vein for the recording of arterial pressure and the administration of drugs, respectively. Arterial pressure was recorded via a Statham pressure transducer (model P23 XL), and a Grass tachograph (model 7P4K) triggered by the arterial pressure pulse was used to monitor HR. Both arterial pressure and HR were recorded continuously on a Grass polygraph (model 79G).

The head of the animal was placed in a Kopf stereotaxic frame and bent downward at an angle 45° to the horizontal meridian. The dorsal surface of the medulla was exposed by partial occipital craniotomy. The dura was cut and reflected laterally, and the caudal floor of the fourth ventricle was exposed by gently removing the vermis of the cerebellum by suction. Nervous tissue was kept moist by physiological saline throughout the experiment.

Single- or double-barreled glass micropipettes (20- to 35-μm tip diameter) were pulled from 5-μl Socorex capillary tubing (Mississauga, ON, Canada). Micropipettes were placed stereotaxically into the caudal NTS region in which Hcrt-1-like Ir was observed, by using oex as a reference point (rostralcaudal: −0.3 to −1.0 mm; mediolateral: 0.3 to 1.0 mm; dorsalventral: −0.3 to −0.9 mm from the dorsal surface).
The microinjection of Hcrt-1 (0.5, 1.0, 2.5, and 5.0 pmol; Phoenix Pharmaceuticals, Mountain View, CA) in 0.9% saline into the NTS was done by the application of pressurized nitrogen pulses controlled by a picospritzer (General Valve; Fairfield, NJ). The injected volume (10–20 nl) was measured by direct observation of the fluid meniscus in the micropipette by using a microscope fitted with an ocular micrometer that allowed a 2-nl resolution. Two to three sites on each side of the NTS in each animal were tested for the effects of Hcrt-1 on mean arterial pressure (MAP) and HR. Control injections of the vehicle saline into similar sites of the NTS were shown not to elicit cardiovascular responses.

Effect of administration of a muscarinic and nicotinic receptor blocker on MAP and HR responses. To determine which components of the autonomic nervous system were involved in mediating cardiovascular responses elicited by Hcrt-1 in NTS, in eight additional animals under either α-chloralose (n = 4) or urethane (n = 4) anesthesia, the cardiovascular responses elicited by the microinjection of Hcrt-1 (1.0 pmol) into the NTS were retested after the systemic injection of the muscarinic receptor blocker atropine methyl bromide (2 mg/kg iv). In five of these animals, the effect of total autonomic blockade was investigated after the intravenous injection of atropine methyl bromide (2 mg/kg) and hexamethonium bromide (20 mg/kg). Only one site in each animal was tested in these studies.

Activation of the baroreceptor reflex. To determine whether Hcrt-1 exerted an effect on the baroreceptor reflex, the effect of microinjection of Hcrt-1 (1.0 pmol) into NTS on the reflex bradycardia elicited by the increase in MAP to an intravenous injection (0.05–0.1 ml) of phenylephrine (PE; 2, 3, or 4 μg/kg) was tested in eight additional animals under urethane anesthesia. Injections of PE were made 5 min before (control) and 0.5, 2.5, and 5.0 min after the microinjection of Hcrt-1 into NTS. In each animal, injections of 2, 3, or 4 μg/kg of PE were made while testing only one site in each side of the NTS.

Histological verification of injection sites. At the end of all experiments, the micropipette was withdrawn from the last site of Hcrt-1 microinjection, emptied of the Hcrt-1 solution, and filled with Pontamine sky blue in 0.9% saline and lowered back stereotaxically to the same site at which a 20-nl microinjection of the dye was made to mark the injection site in the NTS. Animals were perfused with 50 ml of 0.9% saline followed by 50 ml of 10% formalin. Injections of the Pontamine sky blue dye did not elicit cardiovascular responses at the site at which the Hcrt-1 previously elicited a depressor and bradycardia response. Brains were postfixed in the 10% buffered formalin solution for 2–4 days. Frozen transverse sections of the brain stem were cut in a cryostat at 50 μm, mounted on glass slides, and stained with neutral red. Stimulation sites were determined by extrapolation along a pipette tract from the center of the marked injection site. All stimulation sites were mapped on projection drawings of transverse sections of the rat brain stem for each animal and later plotted on a standard set of drawings of sections of the dorsomedial medulla (32).

Data analysis. Means ± SE were calculated for the magnitude of the peak changes in MAP and HR. A response was defined as a change in MAP or HR of ≥5 mmHg or 5 beats/min, respectively. Comparisons of the changes in MAP or HR before and after the administration of the muscarinic or nicotinic receptor blocking agents were made by using the ANOVA for repeated measures, followed by a Bonferroni post hoc test. Effects of Hcrt-1 microinjection on the baroreceptor reflex were analyzed by using a regression analysis and the statistical comparisons among the slopes of the lines were made by using an ANOVA, followed by Dunnett’s multiple-comparison test. In all cases, a P value of < 0.05 was taken to indicate statistical significance.

RESULTS

Hcrt-1 Ir within the NTS region. Figure 1 summarizes the distribution of Hcrt-1-like Ir in the caudal NTS region of the male rat. Hcrt-1 labeling was observed throughout the NTS complex. However, relatively moderate-to-dense fiber and presumptive terminal labeling was found within the dorsolateral (Slz; Fig. 2) and interstitial (Sni) subnuclei of the NTS (Figs. 2, A and B) compared with other subnuclei in the NTS. Within the caudal Slz, presumptive terminal-like labeling was consistently observed in the area just dorsal to the solitary tract (Fig. 2C). Additionally, moderate Hcrt-1 labeling was found in the medial (Sm) subnuclei, whereas the commissural (Com) subnuclei of NTS contained light Hcrt-1 labeling (Figs. 1 and 2, A and B). Although a large amount of Hcrt-1 labeling was also found within the ventral subnucleus of NTS (Figs.
and 2, A and B), these labeled fibers appeared to course through this region enroute to other regions of the NTS complex. Furthermore, a large number of labeled fibers were observed within the subpostremal area (subnu-
cleus gelatinosa of the NTS). Many of these fibers appeared to continue into the area postrema (Figs. 1 and 2A). Of note was the apparent lack of Hcrt-1 Ir within the central subnucleus of the NTS.

Within adjacent medullary structures to NTS that comprise the dorsal vagal complex, it was interesting to note that the dorsal motor nucleus of the vagus (DMV) contained very few labeled fibers (Figs. 1 and 2, A and B). These labeled fibers were observed mainly within the rostral region of DMV, usually near the lateral aspects of the nucleus. In the caudal DMV, Hcrt-1-labeled fibers usually appeared to course through and around the lateral edges of the nucleus enroute to the NTS. The area postrema was also found to contain a relatively small amount of Hcrt-1 labeling. These labeled fibers were predominantly found along the lateral edges of the nucleus (Figs. 1 and 2A).

Cardiovascular effects of microinjection of Hcrt-1 into NTS. To determine the effect of Hcrt-1 in NTS on the MAP and HR, Hcrt-1 was microinjected at four different dosages at histologically verified sites within the Slt and Sm of the NTS complex (Fig. 3). In the anaesthetized rat, baseline HR and MAP were found to be 405.0 ± 7.4 beats/min and 108.9 ± 4.1 mmHg, respectively. Microinjection of Hcrt-1 into the Slt and Sm regions of NTS elicited dose-dependent depressor and bradycardia responses (Figs. 4 and 5), which were of maximal amplitude with 2.5 pmol injections (Figs. 4 and 5). Increasing the dosage of Hcrt-1 to 5.0 pmol did not further increase the magnitude of the MAP or HR responses (Fig. 5). On occasion, injections of 2.5–5.0 pmol of Hcrt-1 into the caudal Com region of the NTS complex elicited small increases in arterial pressure (range, 5–10 mmHg; n = 9) with or without an associated HR response. A representative experiment showing the effect of injecting different dosages of Hcrt-1 into a site within the Slt (photomicrograph in Fig. 3) is shown in Fig. 4.

Injection of Hcrt-1 into other regions of the NTS complex and into areas immediately outside the NTS complex did not elicit any cardiovascular response (Fig. 3). Similarly, injections of the vehicle, 0.9% physiological saline into the Slt or Sm did not elicit significant cardiovascular responses (Fig. 5).

Autonomic nervous system components mediating Hcrt-1 responses. To investigate which peripheral component of the autonomic nervous system contributed to the cardiovascular responses elicited by microinjection of Hcrt-1 (1.0 pmol) into the NTS, the muscarinic receptor blocker atropine methyl bromide was administered intravenously. The depressor response elicited by the Hcrt-1 was significantly attenuated, whereas the bradycardia response was abolished (Fig. 6). Intravenous administration of both atropine methyl bromide and the nicotinic receptor blocker hexamethonium bromide abolished the depressor response elicited by the Hcrt-1 microinjection (Fig. 6).

Effect of Hcrt-1 injections into NTS on the baroreceptor reflex. The effect of Hcrt-1 microinjection (1.0 pmol) into the Slt or Sm on the reflex bradycardia to activation of arterial baroreceptors after an acute rise in
systemic arterial pressure was investigated in the male anesthetized rat. As shown in Figs. 7 and 8, activation of NTS neurons by Hcrt-1 potentiated the reflex decrease in HR to baroreceptor activation. The increase in the gain of the HR component of the baroreceptor reflex was evident by 0.5 min after the Hcrt-1 microinjection (Figs. 7 and 8). By 2.5 min after the Hcrt-1 microinjection into NTS, the gain of the reflex HR response began to return to control values (Figs. 7 and 8). Figure 7 shows a representative experiment and the data from all experiments are summarized in Fig. 8.

**DISCUSSION**

In situ hybridization and immunocytochemical studies have shown that neurons expressing either Hcrt (orexin) mRNA (17) or the Hcrt peptide (30) are located almost exclusively within the lateral and perifornical hypothalamus. A small number of neurons have also been described within the periventricular hypothalamic area. These Hcrt-containing neurons have been shown to contribute extensively to a number of neuronal systems throughout the brain involved in controlling homeostatic mechanisms (10). Within the brain stem, Hcrt-1 and Hcrt-2 Ir has been observed within the dorsal vagal complex (30, 33), an area well known to be involved in the control of cardiovascular function. This study has demonstrated that the relative density of Hcrt-1 labeling was varied within specific subnuclei of the NTS complex. Hcrt-1 labeling was found predominantly within the caudal Slt, Sni, and Sm subnuclei of NTS. This study has also demonstrated that injections of Hcrt-1 into the NTS of the rat elicits a dose-dependent depressor and bradycardia response. Finally, microinjection of Hcrt-1 into the NTS were

![Fig. 3. A series of transverse sections of the dorsal medial medulla extending from 5.0 to 4.4 mm caudal to the interaural line showing the location of histologically verified sites corresponding to the center of Hcrt-1 microinjection in the NTS complex of the rat. The bright-field photomicrograph stained with neutral red at 4.5 mm caudal to the interaural line shows an injection site (arrow) in the dorsolateral (Slt) subnucleus of NTS. Sites eliciting decreases in mean arterial pressure (MAP) and heart rate (HR); ⊗, sites at which Hcrt-1 microinjection did not elicit cardiovascular responses. For clarity, only sites at which 1.0 pmol elicited cardiovascular responses are shown. In addition, not all injection sites are shown in these transverse sections, because many of the injections sites overlapped those marked on the drawings. Calibration mark, 500 μm in line drawings and 100 μm on photomicrograph; AP, area postrema; Com, commissural subnucleus of NTS.](image)

![Fig. 4. Representative HR, arterial pressure (AP), and MAP responses to microinjection of 0.5–2.5 pmol of Hcrt-1 (arrows) into the same site in the dorsolateral subnucleus of Slt the NTS (site shown in photomicrograph of Fig. 3). Calibration mark, 1 min.](image)

![Fig. 5. Bar chart showing the effect of microinjection into NTS of varying amounts of Hcrt-1 (0.5, 1.0, 2.5, and 5.0 pmol) and the vehicle saline on MAP and HR in the rat. Note that maximal effects were elicited at 2.5 pmol of Hcrt-1. All values are means ± SE. Numbers in parentheses indicate NTS sites injected at that specific dose of Hcrt-1.](image)
found to potentiate the decrease in HR to activation of arterial baroreceptors.

Three subnuclei in the NTS complex that received the densest Hcrt-1 projections, the Slt, Sm, and Sni, have been shown previously to receive direct afferent projections from carotid sinus and aortic arch baroreceptors (7). In addition, the caudal Sm and Com has been shown to be densely innervated by afferents arising from carotid chemoreceptors (16). This suggests the possibility that Hcrt-1 neurons in the lateral hypothalamus may have a prominent role in the modulation of the cardiovascular responses evoked by the activation of baroreceptor and/or chemoreceptor reflexes. It is interesting to note that no Hcrt-1 Ir was observed within the central subnucleus, a region of NTS thought to be involved in gastric function (9). However, the DMV was observed to receive a light Hcrt-1 projection. Consistent with this latter observation, Hcrt-1 has been shown to evoke gastric acid secretion (44) and to directly excite DMV motor neurons in vitro (22). The DMV is known to be involved in the parasympathetic regulation of gastrointestinal function (24).

The finding that microinjection of Hcrt-1 into NTS elicited decreases in MAP and HR was unexpected as it has previously been shown that either intracisternal (4) or intraventricular (39) injections of Hcrt-1 in rats evokes an increase in renal sympathetic nerve activity, and an increase in aterial pressure and HR. In addition, a recent study (40) in which large (500 nl) injections of Hcrt were made into the caudal Com region of NTS has also reported increases in aterial pressure and HR. Although the reasons for this discrepancy are not known, it may be related to the large volumes and dosages of Hcrt-1 used in these previous studies (4, 39, 40), because they were several orders of magnitude greater than those used in the present experiments. In fact, in this study, at the higher dosages of Hcrt-1, small pressor responses could be elicited on occasion from some sites in the caudal Com region of NTS, a region of the NTS shown to receive predominantly chemoreceptor afferent fiber projections (7, 16).

Microinjection of Hcrt-1 into the Slt and Sm subnuclei of NTS elicited a bradycardia and depressor response. These responses are consistent with the cardiovascular effects observed during either electrical or glutamate stimulation of these NTS subnuclei (7, 26).
The bradycardia response was shown to be mediated by activation of vagal cardiomotor neurons as the administration of the muscarinic receptor blocker atropine methyl bromide abolished the decrease in HR. The depressor response was most likely the result of a decrease in cardiac output and sympathoinhibition because atropine administration blocked most of the response, whereas the nicotinic receptor blocker hexamethonium bromide abolished the remaining depressor response.

This study has also demonstrated that Hcrt-1 activates a neuronal circuit in the NTS, which potentiates the reflex vagal bradycardia to activation of arterial baroreceptors. Although it is apparent that Hcrt-1 injections into Slt or Sm reset the baroreceptor reflex, a lower level, this finding may also suggest that Hcrt-1 may have altered the gain of the vagal component of the baroreceptor reflex. However, this latter suggestion should be viewed with caution because the entire baroreceptor reflex curve was not examined in this study. It is possible that the Hcrt-1 injections into NTS may have shifted the location of the operating point of the baroreceptor reflex on the reflex curve. Thus the apparent increase in the gain of the reflex may just represent a shift of the operating point to a higher part of the reflex curve. The bolus injection of PE used in this study to reflex activate arterial baroreceptors allowed predominantly for the study of the vagal HR component of the reflex (8). As injections of Hcrt-1 into NTS were shown to elicit only vagal bradycardia, it is unlikely that Hcrt-1 altered central circuits that contributed to decreases in HR as a result of sympathoinhibition, which have also been reported to occur in the conscious animal after activation of the baroreceptor reflex after long-term infusions of pressor agents (8). However, arterial pressure depressor responses were evoked after Hcrt-1 injections into NTS, and these responses were due to decreased sympathetic activity. Therefore, the possibility also exists that Hcrt-1 may have altered the arterial pressure reflex responses to activation of baroreceptors.

The central mechanism by which Hcrt-1 potentiates the baroreflex effect is not known. However, it is interesting to note that Hcrt-1Ir fibers are found in greater concentration in those regions of the NTS, such as the Slt and Sm, in which baroreceptor afferent fibers are known to terminate (7). Therefore, these observations suggest the possibility that Hcrt-1 may have a direct effect on primary baroreceptor afferent fibers. In support of this possibility, it is interesting to note that stimulation of the lateral hypothalamic area has been shown to cause primary baroreceptor afferent depolarization (41). In addition, Hcrt-1 has been reported to facilitate the release of glutamate from nerve terminals (47), the putative transmitter thought to be released by baroreceptor afferent fibers (43). However, the possibility also exists that Hcrt-1-containing fibers are exerting an effect on second-order NTS neurons in the baroreceptor reflex arc, therefore increasing their responsiveness to activation by baroreceptor afferent inputs.

No attempt was made in these studies to identify the central pathways mediating the cardiovascular effects elicited by the Hcrt-1 injections into NTS. However, it is likely that Hcrt-1-activated neurons that primarily projected directly to the nucleus ambiguus (34), the site of origin of vagal cardioinhibitory axons (31), and to a lesser extent, to caudal ventrolateral medullary neurons (5, 34) that inhibit rostral ventrolateral sympathetic premotor neurons (5). This would be consistent with the observation that the bradycardia response is mediated by vagal activation and the depressor response, in part, by sympathoinhibition.

It is interesting to note that stimulation of the lateral and perifornical hypothalamic areas in which Hcrt-1 neurons projecting to the NTS region are found (20) has been shown to elicit depressor and bradycardia responses (1, 18) similar to those evoked in this study from the NTS sites. This suggests the existence of a direct cardiovascular Hcrt-1-containing pathway descending from the hypothalamus to NTS. The fact that we found in this study that Hcrt-1 microinjection potentiated the HR component of the baroreceptor reflex suggests that this descending pathway may also exert a direct effect on NTS neurons that receive baroreceptor afferent inputs and mediate the activation of vagal cardiomotor neurons.

In perspective, Hcrt was originally described to induce feeding behavior when administered into the lateral cerebral ventricles (35). During feeding, a variety of circulatory changes occur that are associated with the motor and autonomic components of ingestion (27). It has been described that during the cephalic phase of the ingestion, arterial pressure increases, whereas during the postprandial period, a vasodilation of the mesenteric vascular bed occurs (27). The observation that microinjection of Hcrt into the rostral ventrolateral medulla, the site of origin of the sympathetic premotor neurons (5), results in an increase in MAP and HR (4) suggests that at this level, orexin systems may be involved in the integration between the motor and the cardiovascular systems related to both obtaining food and the ingestion of the food. On the other hand, the Hcrt projections to the NTS may function to modulate the activity of visceral afferents and/or to provide a general activation of the parasympathetic system during the digestive and absorptive phases of ingestion. However, it is now clear that orexin systems are not only involved in ingestive behaviors, but also exert an effect on a variety of physiological mechanisms involved in homeostasis (3, 13, 19, 21, 23, 25, 42, 44, 45, 48). Therefore, it is possible that orexigenic pathways may function to adjust cardiovascular responses to activation of these different homeostatic mechanisms (10, 12, 15, 33, 42).

In summary, microinjection of Hcrt-1 into the Slt and Sm subnuclei of the NTS elicited a vagal bradycardia and a depressor response as a result of decreased sympathetic activity to the vasculature. In addition, the Hcrt-1 microinjection potentiated the HR component of the baroreflex by altering the excitability of neuronal circuits in NTS that reflexly control the...
circulation. These data suggest that Hcrt-1 circuits in
the brain play an important role in the regulation of
physiological processes controlling the cardiovascular
system.

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