Cardiovascular responses to microinjections of urocortins into the NTS: role of inotropic glutamate receptors

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Nakamura T, Sapru HN. Cardiovascular responses to microinjections of urocortins into the NTS: role of inotropic glutamate receptors. Am J Physiol Heart Circ Physiol 296: H2022–H2029, 2009. First published April 24, 2009; doi:10.1152/ajpheart.00191.2009.—Urocortin 1 (Ucn1) and urocortin 3 (Ucn3) are new members of the corticotrophin-releasing factor (CRF) peptide family. Ucn1 is a ligand for both the CRF type 1 receptors (CRF1Rs) and the CRF type 2 receptors (CRF2Rs), whereas Ucn3 is a high-affinity ligand for the CRF2Rs. Recently, we reported that Ucn3 microinjections into the medial nucleus tractus solitarius (mNTS) elicit decreases in mean arterial pressure (MAP) and heart rate (HR) (Nakamura T, Kawabe K, Sapru HN. Am J Physiol Heart Circ Physiol 296: H325–H332, 2009). The presence of CRF2Rs on afferent terminals has been reported in the mNTS of the rat. It was hypothesized that activation of CRF2Rs on afferent terminals in the mNTS may release glutamate, which, in turn, may elicit decreases in MAP and HR via activation of ionotropic glutamate receptors (iGLURs). This hypothesis was tested in urethane-anesthetized, artificially ventilated, adult male Wistar rats. Microinjections (100 nl) of Ucn1 (0.12 mM) into the mNTS elicited decreases in MAP and HR. The responses were partially blocked by microinjections of iGLUR antagonists into the mNTS. On the other hand, the decreases in MAP and HR elicited by microinjections of Ucn3 (0.06 mM) into the mNTS were completely blocked by microinjections of iGLUR antagonists into the mNTS. These results indicate that activation of CRF2Rs in the mNTS, by Ucn1 and Ucn3, releases glutamate, which, in turn, elicits decreases in MAP and HR via activation of iGLURs.

UROCORTEINS [UROCORTINS 1 (Ucn1), 2 (Ucn2), and 3 (Ucn3)] are members of the corticotrophin-releasing factor (CRF; also known as corticotropin-releasing hormone) peptide family. Two major subtypes of CRF receptors (CRFRs), CRF type 1 receptors (CRF1Rs) and CRF type 2 receptors (CRF2Rs), have been identified, and both types are members of the subfamily of seven-transmembrane receptors that are coupled to G proteins (7, 9, 12, 15). The presence of these receptors in the medial nucleus tractus solitarius (mNTS) has been reported (3, 20).

Recently, we reported that Ucn3 microinjections into the mNTS elicit decreases in mean arterial pressure (MAP) and heart rate (HR) (13). Microinjections of Ucn1 and Ucn2 into the mNTS have also been reported to elicit decreases in MAP and HR (22). It is well established that the mNTS is the site at which baroreceptor, chemoreceptor, and cardiopulmonary afferents make their first synapses (5, 16, 17). The presence of presynaptic CRF2Rs on afferent terminals has been reported in the mNTS of the rat (11). Glutamate has been reported to be the main neurotransmitter in baroreceptor, chemoreceptor, and cardiopulmonary receptor terminals in the mNTS (16, 17). Based on this information, it was hypothesized that microinjections of urocortins into the mNTS may activate the presynaptic CRF2Rs, releasing glutamate, which, in turn, may elicit decreases in MAP and HR via activation of iGLURs (8). The hypothesis was tested in rats, using Ucn1, which mediates its actions via both CRF1Rs and CRF2Rs, and Ucn3, which mediates its actions via CRF2Rs (12). There is no report in the literature in which the role of glutamate receptors in mediating cardiovascular responses to urocortins in the mNTS has been studied.

MATERIALS AND METHODS

General procedures. Experiments were done in adult male Wistar rats (Charles River Laboratories, Wilmington, MA) weighing 300–360 g (n = 73). All animals were housed under controlled conditions with a 12:12-h light-dark cycle. Food and water were available to the animals ad libitum. The experiments were performed according to the National Institutes of Health “Guide for the Care and Use of Laboratory Animals” (7th Ed., 1996) and with the approval of the Institutional Animal Care and Use Committee of this university.

The general procedures have been described in detail elsewhere (13). Briefly, one of the veins and trachea were cannulated under inhalation anesthesia with isoflurane. Urethane (1.2–1.4 g/kg) was injected intravenously in divided doses, and isoflurane anesthesia was terminated. The absence of a pressor response and/or withdrawal of the limb in response to pinching of a hind paw indicated that the rats were properly anesthetized. The rats were artificially ventilated, and end-tidal CO2 was maintained at 30–35 mmHg. Rectal temperature was maintained at 37.0 ± 0.5°C. Blood pressure and HR were recorded by standard techniques.

Microinjections. The details of this technique are described elsewhere (13). Briefly, the rats were placed in a prone position in a stereotaxic instrument with bite bar 18 mm below the interaural line. The microinjections were made using four-barreled glass micropipettes (tip size 20–40 μm). The coordinates for the mNTS were 0.5–0.6 mm rostral and 0.5–0.6 lateral to the calamus scriptorius (CS) and 0.5–0.6 mm deep from the dorsal medullary surface. The volumes of all microinjections into the mNTS were 100 nl (10). The duration of microinjection was 5–10 s. Microinjections (100 nl) of artificial cerebrospinal fluid (aCSF, pH 7.4) or 20% dimethyl sulfoxide (DMSO) (pH 7.4; see Drugs and chemicals) into the mNTS were used as controls.

Greater splanchnic nerve recording. The greater splanchnic nerve (GSN) was exposed retroperitoneally and sectioned at its junction with the celiac ganglion; a few millimeters of the central end were desheathed; and whole nerve activity was recorded by standard techniques (13, 18). At the end of the experiment, the GSN was sectioned centrally, and the remaining activity was considered to be the noise level.
Histology. Typical sites of microinjections in the mNTS were marked by microinjections of diluted India ink (100 nl). The animals were perfused and fixed with 4% paraformaldehyde; serial sections of the medulla were cut (40 μm) and stained with cresyl violet. The microinjection sites were identified using a standard atlas (14).

Drugs and chemicals. The following drugs and chemicals were used: (d-L)-2-amino-7-phosphono-heptanoic acid (d-AP7; NMDA receptor antagonist), 2,3-dioxo-6-nitro-1,2,3,4-tetrahydropenzo-(f)quinoxaline-7-sulfonamide disodium (NBQX disodium salt; non-NMDA receptor antagonist), 5-chloro-N-(cyclopropylmethyl)-2-methyl-N-propyl-N’-(2,4,6-trichlorophenyl)-4,6-pyrimidinediamine hydrochloride (NBI 27914; selective antagonist for CRF1R) (279), K41498 (selective antagonist for CRF2R) (11), Ucn1, Ucn3, l-glutamate monosodium (l-Glu), carbachol (Carb), l-phenylephrine hydrochloride, isoflurane, and urethane. A solution of NBI 27914 was freshly prepared in aCSF (298 ± 2 mosmol/kgH2O; pH 7.4). Where applicable, the concentration of drugs refers to their salts. The sources of different drugs and chemicals were as follows: Ucn1 and Ucn3 (American Peptide, Sunnyvale, CA), NBI 27914 and K41498 (Tocris Cookson, Ellisville, MO), and isoflurane (Baxter Pharmaceutical Products, Deerfield, IL). All other drugs and chemicals were obtained from Sigma Chemicals (St. Louis, MO).

Statistical analyses. Means and SEs were calculated for maximum changes in MAP and HR. In the concentration-response studies, maximum decreases in MAP and HR in different groups of rats were compared by one-way ANOVA followed by Tukey-Kramer’s multiple comparison. In experiments testing for tachyphylaxis and effect of CRFR antagonists or iGLUR antagonists, comparisons of the maximum decreases in MAP and HR elicited by microinjection of Ucn1 or Ucn3 into the mNTS were made by repeated-measures ANOVA followed by Tukey-Kramer’s multiple-comparison test. Student’s paired t-test was used for all other statistical analyses. For the analysis of nerve activity, control value represented the average amplitude of integrated GSNA activity (GSNA) during 35-s period before the intravenous administration or the microinjections of drugs. Maximum inhibition in GSNA amplitude, in response to intravenous administration or microinjections of drugs into the mNTS, was expressed as percent decrease from the control value of GSNA amplitude. The mean values of the integrated nerve signals were compared using Student’s paired t-test. In all cases, the differences were considered significant at P < 0.05.

RESULTS

Baseline values for MAP and HR in urethane-anesthetized rats were 99.5 ± 2.4 mmHg and 410.0 ± 10.9 beats/min, respectively (n = 73).

Concentration response of Ucn1. In this series of experiments, the mNTS was identified by microinjections of l-Glu (5 mM), which stimulates neurons, but not fibers of passage. Microinjections of l-Glu into the mNTS elicited decreases in MAP and HR responses. The interval between the microinjections of l-Glu and other agents was at least 5 min. Microinjections (100 nl) of Ucn1 (0, 0.06, 0.12, 0.25 mM) into the mNTS elicited decreases in MAP (0.8 ± 0.5, 11.4 ± 3.0, 20.5 ± 2.1, and 17.5 ± 2.1 mmHg, respectively) and HR (0.5 ± 0.5, 8.5 ± 1.4, 15.0 ± 3.9, and 9.1 ± 2.3 beats/min, respectively) (n = 9) (Fig. 1). The maximal decreases in MAP and HR were elicited by 0.12 mM concentration of Ucn1. The onset and duration of cardiovascular responses to microinjections of Ucn1 (0.12 mM) were 1–5 s and 60–120 s, respectively. The peak effect was observed at 10–60 s.

The decreases in MAP in response to three consecutive microinjections of Ucn1 (0.12 mM) were 17.1 ± 1.7, 18.0 ± 2.7, and 18.5 ± 2.3 mmHg, respectively and the decreases in HR were 17.5 ± 2.8, 20.8 ± 2.3, and 19.5 ± 3.7 beats/min, respectively (n = 6) (P < 0.05), indicating that Ucn1 microinjections at 40-min intervals did not exhibit tachyphylaxis. Therefore, the interval between the microinjections of Ucn1 was at least 40 min in all experiments.

Site specificity of Ucn1-induced responses. The site specificity of Ucn1-induced responses was tested in another group of rats (n = 5). First, the mNTS site was identified by microinjections of l-Glu, and Ucn1 (0.12 mM) was microinjected at the same site to elicit usual decreases in MAP and HR. Then the same concentrations of l-Glu and Ucn1 were microinjected into an adjacent site (e.g., the cuneate nucleus; 0.5 mm rostral and 1.5 mm lateral to the CS; and 0.5 mm ventral to the dorsal surface of the medulla). Neither l-Glu, nor Ucn1 elicited any cardiovascular response at this site.

Effect of Ucn1 on sympathetic nerve activity. The effect of microinjections of Ucn1 into mNTS on efferent sympathetic nerve activity was also studied (n = 5). Tracings of a typical recording are shown in Fig. 2. Efferent discharge was recorded from a segment of the GSN. An intravenous bolus injection of phenylephrine (PE; 10 μg/kg) increased MAP, which, in turn, elicited reflex bradycardia and inhibition of efferent sympathetic nerve discharge (Fig. 2A). About 10 min later, when the effects of PE subsided, microinjection of Carb (0.5 mM) into the mNTS elicited an inhibition of the sympathetic nerve discharge, which lasted for 30–120 s (Fig. 2B). After 5 min, microinjection of aCSF (100 nl) into the same mNTS site did not alter the sympathetic nerve activity (not shown). Within 2 min, Ucn1 (0.12 mM) was microinjected at the same site; a decrease in efferent nerve discharge was elicited that lasted for 10–100 s (Fig. 2C).

In group data for splanchnic nerve, an intravenous bolus injection of PE (10 μg/kg) elicited significant (P < 0.05) decreases in the GSNA (83.9 ± 3.6%) compared with basal nerve activity. Microinjection of Carb (0.5 mM) and Ucn1 (0.12 mM) into the mNTS elicited significant (P < 0.05) decreases in the GSNA (67.6 ± 2.9% and 92.8 ± 0.5%, respectively).
Fig. 2. Effect of microinjections of Ucn1 into mNTS on splanchnic nerve activity. First trace: MAP (mmHg); second trace: pulsatile arterial pressure (PAP, mmHg); third trace: HR (bpm); fourth trace: integrated greater splanchnic nerve activity (fGSNA; μV/50 ms); and fifth trace: whole GSNA (μV). Reflex inhibition of GSNA elicited by pressor response induced by phenylephrine (PE; 10 μg/kg iv; A) and microinjection of carbachol (Carb; 0.5 mM; B) and Ucn1 (0.12 mM; C) into the mNTS are shown. In group data for this experiment, an iv bolus injection of PE (10 μg/kg) and microinjections of Carb (0.5 mM) or Ucn1 (0.12 mM) into the mNTS elicited significant (P < 0.05) decreases in the GSNA compared with the basal nerve activity.

decreases in the GSNA (83.7 ± 1.3 and 55.8 ± 5.4%, respectively) compared with basal nerve activity.

Effect of CRF1R antagonists on Ucn1-induced response. The effects of a selective CRF1R antagonist (NBI 27914) on the cardiovascular responses to microinjections of Ucn1 into the mNTS were studied as follows (n = 5). A site in the mNTS was identified on one side by microinjection of L-Glu (5 mM), and initial decreases in MAP and HR responses (27.8 ± 1.1 mmHg and 31.0 ± 2.9 beats/min, respectively) to Ucn1 (0.12 mM) were recorded. Within 40 min, NBI 27914 (1.5 mM, 100 nl) was microinjected at the same site. The concentration of NBI 27914 used (1.5 mM) was selected because one-half of this dose (0.75 mM) did not alter Ucn1-induced cardiovascular responses. Microinjections of NBI 27914 alone into the mNTS did not elicit a cardiovascular response. However, the vehicle in which it was dissolved (20% DMSO) elicited decreases in MAP and HR (28.4 ± 6.8 mmHg and 64.0 ± 19.4 beats/min, respectively), which lasted for 60–180 s. Five minutes after the microinjection of NBI 27914, when the MAP and HR returned to a basal level, Ucn1 (0.12 mM) was again microinjected at the same site; the decreases in MAP and HR (16.8 ± 1.1 mmHg and 13.0 ± 3.3 beats/min, respectively) to Ucn1 were attenuated (P < 0.01). Sixty minutes after the blockade of CRF1R in the mNTS, the Ucn1-induced decreases in MAP and HR (24.2 ± 2.4 mmHg and 32.0 ± 6.6 beats/min, respectively) recovered at initial levels (Fig. 3A). In the same group of rats, NBI 27914 did not alter the decreases in MAP and HR to L-Glu. The decreases in MAP to microinjections of L-Glu (5 mM) before and 8 min after the microinjections of NBI 27914 were 40.8 ± 3.6 and 39.8 ± 2.8 mmHg (P > 0.05), respectively. Similarly, the bradycardic responses to microinjections of L-Glu (5 mM) before and 8 min after the microinjections of NBI 27914 were 78.0 ± 17.7 and 72.0 ± 17.7 beats/min (P > 0.05), respectively.

Ucn1-induced decreases in MAP before and 5 min after the microinjections of vehicle (20% DMSO) were 21.6 ± 1.9 and 20.0 ± 1.9 mmHg, respectively, and the decreases in HR were 27.0 ± 4.6 and 31.0 ± 5.0 beats/min, respectively (P > 0.05), indicating that the vehicle did not alter Ucn1-induced responses (n = 5).

Effect of CRF2R antagonists on Ucn1-induced response. The effects of a selective CRF2R antagonist (K41498) on the cardiovascular responses to microinjections of Ucn1 into the mNTS were studied as follows (n = 4). A site in the mNTS was identified on one side by microinjection of L-Glu (5 mM), and initial decreases in MAP and HR (21.5 ± 2.2 mmHg and 32.5 ± 5.9 beats/min, respectively) to microinjection of Ucn1 (0.12 mM) were recorded. Within 40 min, K41498 (5 mM, 100 nl) was microinjected at the same site. The concentration of K41498 used (5 mM) was selected based on our previous report (13). The decreases in MAP and HR (11.0 ± 0.5 mmHg and 13.5 ± 1.1 beats/min, respectively) to Ucn1 microinjected 5 min after K41498 were attenuated (P < 0.01). The baseline MAP and HR before (104.0 ± 4.0 mmHg and 397.5 ± 13.3 beats/min, respectively) and after (100.0 ± 4.0 mmHg and 390.0 ± 10.2 beats/min, respectively) the CRF2R blockade in the mNTS were not statistically different (P > 0.05). Sixty minutes after the blockade of CRF2Rs in the mNTS, the
Ucn1-induced decreases in MAP and HR (15.0 ± 2.0 mmHg and 17.5 ± 1.4 beats/min, respectively) did not recover completely (Fig. 3B). In the same group of rats, K41498 did not alter the decreases in MAP and HR to microinjections of l-Glu (5 mM); l-Glu-induced decreases in MAP before and 8 min after the microinjections of K41498 were 39.2 ± 3.9 and 38.5 ± 4.0 mmHg (P > 0.05), respectively. Similarly, l-Glu-induced decreases in HR before and 8 min after the microinjections of K41498 were 56.2 ± 19.5 and 43.7 ± 12.8 beats/min, respectively (P > 0.05).

Effect of combined CRF₁R and CRF₂R antagonist on Ucn1-induced response. The effect of combined blockade of CRF₁Rs and CRF₂Rs in the mNTS on the cardiovascular responses elicited by microinjections of Ucn1 (0.12 mM) into the mNTS was studied as follows (n = 5). The mNTS site was identified on one side by microinjection of l-Glu (5 mM). The initial decreases in MAP and HR by microinjection of Ucn1 (0.12 mM) into the mNTS were 27.6 ± 2.5 mmHg and 27.0 ± 7.0 beats/min, respectively. After an interval of 40 min, NBI 27914 (1.5 mM, 100 nl) and K41498 (5 mM, 100 nl) were microinjected at the same site. The microinjection of Ucn1 (0.12 mM) was repeated at the same site within 5 and 60 min. Ucn1-induced decreases in MAP and HR (4.0 ± 1.0 mmHg and 3.4 ± 0.9 beats/min, respectively) were almost completely blocked with the microinjections of NBI 27914 and K41498.

Sixty minutes later, Ucn1-induced decreases in MAP and HR (20.0 ± 1.6 mmHg and 18.0 ± 4.0 beats/min, respectively) showed recovery (Fig. 3C). In the same group of rats, the decreases in MAP and HR to microinjections of l-Glu (5 mM) were not significantly (P > 0.05) altered by NBI 27914 and K41498. The decreases in MAP to microinjections of l-Glu (5 mM) before and 8 min after the microinjections of NBI 27914 and K41498 were 41.0 ± 2.3 and 39.2 ± 3.3 mmHg, respectively. Similarly, the bradycardic responses to microinjections of l-Glu (5 mM) before and 8 min after the microinjections of NBI 27914 and K41498 were 59.0 ± 11.0 and 54.0 ± 11.3 beats/min, respectively.

Effect of iGLUR antagonists on Ucn1-induced response. In these experiments, Carb (0.5 mM) was used to identify mNTS, instead of l-Glu, because iGLUR antagonists do not alter responses to Carb. The initial decreases in MAP and HR by microinjection of Ucn1 (0.12 mM) into the mNTS were 19.6 ± 2.2 beats/min, respectively. After an interval of 40 min, NBQX (2 mM, 50 nl) and D-AP7 (5 mM, 50 nl) were microinjected at the same site. The concentrations of NBQX and D-AP7 were selected based on our laboratory’s previous reports (8, 10). The microinjection of Ucn1 (0.12 mM) was repeated at the same site within 5 and 60 min. Ucn1-induced decreases in MAP and HR (8.0 ± 0.5 mmHg and 11.4 ± 2.2 beats/min, respectively) were signifi-
significantly attenuated \((P < 0.01)\) by NBQX and D-AP7, but were not completely blocked. Sixty minutes after the blockade of iGLURs, the Ucn1-induced decreases in MAP and HR \((11.2 \pm 1.8 \text{ mmHg} \text{ and } 16.0 \pm 2.4 \text{ beats/min, respectively})\) did not completely recover to initial levels (Fig. 4A). In the same group of rats, the decreases in MAP and HR responses to microinjections of Carb \((0.5 \text{ mM})\) were not significantly \((P > 0.05)\) altered by NBQX and D-AP7. The decrease in MAP to microinjections of Carb \((0.5 \text{ mM})\) before and 8 min after the microinjections of NBQX and D-AP7 were 35.0 \pm 1.5 and 37.0 \pm 2.0 mmHg, respectively. Similarly, the bradycardic responses to microinjections of Carb \((0.5 \text{ mM})\) before and 8 min after the microinjections of NBQX and D-AP7 were 46.0 \pm 10.1 and 47.4 \pm 9.0 beats/min, respectively.

Effect of combined iGLUR and CRF1R antagonist on Ucn1-induced response. The initial decreases in MAP and HR by microinjection of Ucn1 \((0.12 \text{ mM})\) into the mNTS were 23.8 \pm 1.7 mmHg and 22.3 \pm 3.8 beats/min, respectively \((n = 6)\). The initial microinjection of Ucn1 \((0.12 \text{ mM})\) into the mNTS, within 40 min, NBQX \((2 \text{ mM}, 50 \text{ nl})\), D-AP7 \((5 \text{ mM}, 50 \text{ nl})\), and NBI 27914 \((1.5 \text{ mM}, 100 \text{ nl})\) were microinjected at the same site. The microinjection of Ucn1 \((0.12 \text{ mM})\) was repeated at the same site within 5 and 60 min. Ucn1-induced decreases in MAP and HR \((1.8 \pm 0.8 \text{ mmHg} \text{ and } 0.5 \pm 0.5 \text{ beats/min, respectively})\) were complete blocked by prior microinjections of NBQX, D-AP7, and NBI 27914. Complete recovery of Ucn1-induced decreases in MAP and HR \((12.5 \pm 2.1 \text{ mmHg} \text{ and } 12.5 \pm 1.7 \text{ beats/min, respectively})\) was not observed, even 60 min after the blockade of iGLURs and CRF1Rs in the mNTS (Fig. 4B). In the same group of rats, the decreases in MAP and HR to microinjections of Carb \((0.5 \text{ mM})\) were not significantly \((P > 0.05)\) altered by NBQX, D-AP7, and NBI 27914. The decrease in MAP to microinjections of Carb \((0.5 \text{ mM})\) before and 8 min after the microinjections of NBQX, D-AP7, and NBI 27914 were 44.6 \pm 2.2 and 42.0 \pm 2.1 mmHg, respectively. Similarly, the bradycardic responses to microinjections of Carb \((0.5 \text{ mM})\) before and 8 min after the microinjections of NBQX, D-AP7, and NBI 27914 were 27.5 \pm 4.4 and 27.5 \pm 3.5 beats/min, respectively.
and D-AP7 were 35.0 ± 11.7 and 36.6 ± 11.4 beats/min, respectively. The microinjection of NBQX and D-AP7 alone did not exert any effect on basal MAP and HR.

In another group of rats (n = 4), effect of NMDA receptor antagonist (D-AP7) on Ucn3-induced responses was studied. The initial decreases in MAP and HR by microinjection of Ucn3 (0.06 mM) into the mNTS were 22.5 ± 2.1 mmHg and 21.2 ± 1.2 beats/min, respectively. Five minutes after the microinjection of D-AP7 (5 mM) into the mNTS, the Ucn3-induced decreases in MAP and HR were 10.2 ± 1.0 mmHg and 9.2 ± 0.4 beats/min. Thus Ucn3-induced decreases in MAP and HR were significantly (P < 0.01) reduced by microinjections of D-AP7. The effect of non-NMDA receptor antagonist (NBQX) on Ucn3-induced response was also studied (n = 4). The initial decreases in MAP and HR by microinjection of Ucn3 (0.06 mM) into the mNTS were 23.0 ± 1.8 mmHg and 19.7 ± 2.0 beats/min, respectively. Five minutes after the microinjection of NBQX (2 mM) into the mNTS, the Ucn3-induced decreases in MAP and HR were 12.0 ± 1.4 mmHg and 7.5 ± 1.4 beats/min, indicating that Ucn3-induced decreases in MAP and HR were significantly reduced by microinjections of NBQX (P < 0.01 and P < 0.05, respectively).

Histology. The mNTS sites, where microinjections of L-Glu elicited decreases in MAP and HR, were marked in eight rats; a typical mNTS site marked with India ink (100 nl) is shown in Fig. 6A. Figure 6, B and C, represents composite diagrams of mNTS sites; the sites were located 0.5–0.6 mm rostral to the CS.

DISCUSSION

The main finding of this study is that activation of CRF2Rs in the mNTS elicits decreases in MAP and HR that are mediated via iGLURs. Activation of CRF2Rs was accomplished by microinjections of Ucn3, which is believed to be an endogenous ligand for these receptors (12). We have previously reported that microinjections of Ucn3 into the mNTS in unanesthetized, decerebrate, and urethane-anesthetized rats elicit changes in MAP and HR (13). It is also well established that microinjections of glutamate into the mNTS elicit decreases in MAP and HR (17, 19). The similarity of cardiovascular responses to microinjections of Ucn3 and glutamate in the mNTS prompted us to test whether Ucn3-induced responses were mediated via glutamate release. It is well known that the terminals of baroreceptor, chemoreceptor, and cardiopulmonary receptors make their first synapse in the mNTS, and the main neurotransmitter in these terminals is glutamate (5, 16, 17). The presence of presynaptic CRF2Rs on afferent terminals has been reported in the mNTS of the rat (11). In this report (11), unilateral nodose ganglionectomy reduced CRF2R binding in the ipsilateral NTS by 65% compared with the contralateral NTS. The remaining CRF2R binding in the ipsilateral NTS may be on either vagal afferents projecting from the contralateral intact vagus or on nonvagal afferent terminals. It is, therefore, likely that Ucn3 activated CRF2Rs on the afferent nerve terminals in the mNTS and released glutamate, which, in turn, elicited decreases in MAP and HR by activating both NMDA and non-NMDA receptors on the secondary mNTS neurons. Consistent with these results is our observation that microinjections of iGLUR antagonists completely blocked the effects of Ucn3 in the mNTS, and individual microinjections of NMDA or non-NMDA receptor antagonists partially blocked the effects of Ucn3 in the mNTS.

The decreases in MAP and HR induced by microinjections of Ucn1 into the mNTS were also partly mediated via iGLURs. This conclusion was based on our observation that iGLUR
antagonists attenuated, but did not completely block, the Ucn1-induced decreases in MAP and HR. Ucn1 has been reported to activate both CRF1Rs and CRF2Rs (12). Ucn1, like Ucn3, may have activated CRF2Rs on the afferent terminals and released glutamate, which, in turn, elicited decreases in MAP and HR. Consistent with this conclusion is the report that activation of CRF2Rs, but not CRF1Rs, released glutamate in other brain areas (e.g., the ventral tegmental area) (21). Since iGLUR antagonists blocked the Ucn1-induced decreases in MAP and HR only partially, the remaining responses were probably mediated via activation of CRF2Rs located on the secondary mNTS neurons. In this context, it may be noted that application of Ucn1 directly by brief pressure pulses has been reported to increase the firing rate of cerebellar Purkinje neurons in the rat (2).

The specificity of the iGLUR antagonists used in this study was previously established in this laboratory (8, 10). The blockade of Ucn3 or attenuation of Ucn1-induced cardiovascular responses after microinjections of iGLUR antagonists did not recover completely within 60 min, although it showed some recovery. The lack of complete recovery of Ucn1 and Ucn3 responses cannot be attributed to damage to NTS neurons, because responses to other agonists (e.g., Carb) remained unaltered. The lack of complete recovery may be due to persistent blockade of iGLURs in this region, and complete recovery may need >60 min.

Although cardiovascular effects of Ucn1 in the mNTS have been reported previously (22), additional experiments were done to obtain information that was not available in the previous report. In agreement with the previous report (22), we confirmed that, like Ucn3, microinjections of Ucn1 into the mNTS elicited decreases in MAP and HR. We also established that Ucn1-induced decrease in MAP was mediated via inhibition of sympathetic nerve activity. We have previously shown that urocortins mediate bradycardic responses via activation of the vagus nerves (13). In concentration-response studies, microinjections of Ucn1 into the mNTS showed nonlinear bell-shaped curves for MAP and HR. This type of concentration response has been reported for Ucn3 and other peptides (10, 13). This type of response has been explained by homotropic allosterly, in which the agonist at higher concentrations binds to a modulator site, which is different from the primary binding site, and thereby affects the function of the receptor, resulting in attenuated responses (1). The possibility that Ucn1-induced cardiovascular effects were due to its leakage from the microinjection site into the peripheral circulation was excluded because the doses of Ucn1 that elicited decreases in MAP and HR when microinjected into the mNTS did not elicit responses when injected intravenously. The site specificity of Ucn1-induced cardiovascular responses was established by the lack of responses to Ucn1 microinjections into the areas located adjacent to mNTS, such as the cuneate nucleus. Consistent with our previous observations (13), local distortion of brain tissue or any nonspecific effects were not responsible for Ucn1-induced cardiovascular responses, because microinjections of aCSF into the mNTS did not elicit any responses.

Ucn3-induced decreases in MAP and HR were relatively greater compared with the same responses elicited by Ucn1. Perhaps Ucn3-induced glutamate release is greater than that elicited by Ucn1. Glutamate is known to elicit relatively robust decreases in MAP and HR in the mNTS. However, we have no data to support this hypothesis.

Specific and potent antagonists for CRF1Rs (NBI 27914) and CRF2Rs (K41498) were used to demonstrate that Ucn1 effects in the mNTS were mediated via both CRF1Rs and CRF2Rs. Microinjections of the CRF1R antagonist (NBI 27914 and K41498) did not exert any deleterious effects at the site of injection, because they did not alter responses to another unrelated agonist, 1-Glu. Microinjections of NBI 27914 (CRF1R antagonist) and K41498 (CRF2R antagonist) alone into the mNTS did not elicit any cardiovascular responses, suggesting that CRF1Rs and CRF2Rs were not tonically active under normal physiological situations.

The physiological significance of our results can be only speculated at this stage. Stress is a common risk factor in cardiovascular disease, especially hypertension. Stress is known to increase blood pressure in experimental rat models. CRF is one of the neuropeptides that has been implicated in cardiovascular responses to stress (6). Ucn3 is a relatively recent and new addition to the CRF family of peptides, and it mediates its actions via CRF2 receptors (12). The role of these receptors in mediating stress responses is far from clear. In the present paper, we have described the mechanism by which Ucn3 exerts its cardiovascular action in the mNTS. The importance of NTS in cardiovascular regulation, especially reflex regulation, is well established. The results of the present study are likely to provide a baseline on which future studies can be designed in which the role of CRFRs in cardiovascular regulation in normal and pathological states can be delineated. In this paper, we have shown that Ucn3 may release glutamate in the mNTS. It is well established that, in the mNTS, glutamate is the main neurotransmitter for baroreceptor afferent fibers (17). It is, therefore, possible that Ucn3 may modulate baroreflex function at the level of the mNTS.

In conclusion, activation of CRF2Rs in the mNTS by microinjections of Ucn3 elicited decreases in MAP and HR, which were mediated via iGLURs. On the other hand, decreases in MAP and HR to microinjections of Ucn1 into the mNTS were only partially mediated via iGLURs.

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