Bradycardic effects of microinjections of urocortin 3 into the nucleus ambiguus of the rat

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Chitravanshi VC, Kawabe K, Sapru HN. Bradycardic effects of microinjections of urocortin 3 into the nucleus ambiguus of the rat. Am J Physiol Regul Integr Comp Physiol 303: R1023–R1030, 2012.—The presence of urocortin 3 (UCN3) and CRF2 receptors (CRF2R) has been demonstrated in brain tissue. Nucleus ambiguus (nAmb) is the predominant brain area providing parasympathetic innervation to the heart. On the basis of these reports, it was hypothesized that activation of CRF2R in the nAmb may elicit cardiac effects. Experiments were carried out in urethane-anesthetized, artificially ventilated, and adult male Wistar rats. Microinjections of L-glutamate (L-GLU, 5 mM) were used to identify the nAmb. Different concentrations of UCN3 (0.031, 0.062, 0.125, 0.25, and 0.5 mM) microinjected into the nAmb elicited decreases in heart rate (HR) (5.3 ± 1.22 ± 3.3, 38 ± 4.9, 45.7 ± 2.7, and 27.3 ± 2.3 bpm, respectively). The volume of all microinjections was 30 nl. Blood pressure changes concomitant with decreases in HR were not observed. Bradycardia elicited by microinjections of UCN3 (0.25 mM; maximally effective concentration) into the nAmb was significantly (P < 0.05) attenuated by microinjections of selective CRF2R antagonists (K41498, 0.5 mM, and astressin 2B, 0.25 mM) at the same site. Bilateral vagotomy abolished the bradycardic responses to UCN3. These results indicate that activation of CRF2Rs in the nAmb by UCN3 elicited bradycardia, which was vagally mediated. UCNs have been reported to exert cardioprotective effects in heart failure and ischemia/reperfusion injury. In this situation, centrally induced bradycardia by UCN3 would be beneficial. The results of the present investigation provide a platform for future studies on the role of CRF2Rs in the nAmb in pathological states such as heart failure.

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tial volume and frequency were adjusted on the ventilator to maintain the end tidal CO$_2$ at 3.5–4.5%. The rectal temperature was maintained at 37 ± 0.5°C using a rectal probe (RET-1) connected to TCAT-2A temperature controller (Physitemp Instruments, Clifton, NJ). All of the tracings were stored on a computer hard drive. At the end of the experiment, the rats were deeply anesthetized with a high dose of urethane (1.8–2 g/kg iv), a pneumothorax was produced by an incision in one of the intercostal muscles, and cessation of heart beat, which was recorded on line, indicated that euthanasia was complete.

Bilateral vagotomy. In these experiments, the vagus nerves were identified, and loose silk threads were placed around them. The end of the experiment, the rats were deeply anesthetized with a high dose of urethane (1.8–2 g/kg iv), a pneumothorax was produced by an incision in one of the intercostal muscles, and cessation of heart beat, which was recorded on line, indicated that euthanasia was complete.

Microinjection technique. We have described this technique in detail in one of our earlier publications (12). The rats were fixed in a stereotaxic instrument, in a prone position, with a bite bar 18 mm below the interaural line. Multibarreled glass micropipettes (tip size 20–40 μm) were used for microinjections into the nAmb using a dorsal approach. The coordinates for the nAmb were 0.12 caudal to 0.64 mm rostral to obex, 1.8–2 mm lateral to the midline, and 2–2.4 mm below the dorsal surface of the medulla. Controls for microinjections consisted of artificial cerebrospinal fluid (aCSF; pH 7.4). The volume of all microinjections into the nAmb was 30 nl unless indicated otherwise.

Intravenous administration of UCN3. One of the femoral veins of these rats was cannulated, and the concentration of UCN3 (0.25 mM, 30 nl) that elicited maximum responses when microinjected into the nAmb was administered intravenously.

Immunohistochemistry. Surgical procedures for immunohistochemical studies were done under aseptic conditions. The rats, anesthetized with pentobarbital sodium (50 mg/kg ip), were placed prone in a stereotaxic assembly. Unilateral microinjections of colchicine (120 μg, 10 μl) were made into the lateral ventricle (coordinates for the lateral ventricle: 0.8–0.9 mm caudal to the bregma, 1.7–1.8 mm lateral to the midline, and 3.8–4.0 mm deep from the dura). Subcutaneous injections of an antibiotic (cefazolin, 0.05 mg/kg) were administered twice a day to prevent infections. The rats were fixed in a chemical solution (40 °C) in a vibratome (1000 Plus Sectioning System, The Vibratome, St. Louis, MO). The sections containing the nAmb were used for immunostaining for CRF2R and UCN3. The sections were rinsed for 10 min each with 0.1 M PBS. The endogenous peroxidase was then blocked with 3% hydrogen peroxide in 0.1 M PBS (10 min), and the sections were then washed 4 times in 0.1 M PBS, 10 min each.

For CRF2R immunostaining, the sections were incubated with 10% normal rabbit serum (NRS) in 0.1 M PBS containing 0.3% Triton-X (TPBS) for 60 min at room temperature to reduce nonspecific binding of antibodies. The sections were then rinsed for 10 min with 0.1 M PBS and incubated overnight at 4°C with the primary antibody for CRF2R (host goat; 1:100; sc-20550; Santa Cruz Biotechnology, Santa Cruz, CA; diluted with TPBS containing 1% NRS). The sections were again rinsed with PBS, incubated for 2 h at room temperature with the secondary antibody (Cy3-rabbit anti-goat IgG; 1:200, no. 305–165-003, $A_{\text{max}} = 550$ nm, $E_{\text{max}} = 570$ nm; Jackson ImmunoResearch Laboratories, West Grove, PA; diluted with TPBS containing 3% NRS). For UCN3 immunoreactivity, the sections were incubated with 10% normal goat serum (NGS) in TPBS for 60 min at room temperature to reduce nonspecific binding of antibodies. The sections were then incubated overnight at 4°C with the primary antibody for UCN3 (host rabbit; 1:500; H-019–29; Phoenix Pharmaceuticals, Burlingame CA; diluted with TPBS containing 3% NGS). The sections were then rinsed with PBS, incubated for 2 h at room temperature with the secondary antibody (Cy3-goat anti-rabbit IgG; 1:200, no. 111–165-003, $A_{\text{max}} = 550$ nm, $E_{\text{max}} = 570$ nm, Jackson ImmunoResearch Laboratories; diluted with TPBS containing 3% NGS).

In each experiment, the sections were rinsed in PBS after the incubation with the primary and secondary antibodies was completed. The sections were then placed on subbed slides, mounted with Citifluor medium (Ted Pella, Redding, CA), and coverslipped. Laser-scanning confocal microscopy was done to capture the images of the sections, 1 μm apart, using an AIR confocal microscope (Nikon Instruments, Melville, NY).

Histological identification of microinjection sites. Microinjection sites were marked by microinjections of diluted (1:50) green retrograde (Lumafluor, Durham, NC). The animals were perfused and fixed with 2% paraformaldehyde; serial sections of the medulla were cut (40 μm), mounted on slides, covered with Citifluor mountant media, and coverslipped. The microinjection sites were identified, using a fluorescence microscope, photographed, and compared with a standard atlas (34).

Drugs and chemicals. The following drugs and chemicals were used. Astressin 2B, colchicine, d-AP7 (d–(±)-2-amino-7-phosphono-heptanoic acid; N-methyl-D-aspartic acid (NMDA) receptor antagonist), disodium (2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo-[f]quinoline-7-sulfonamide disodium (NBQX); non-NMDA receptor antagonist), isoflurane, gabazine (SR95531), K41498, l-glutamate monosodium ((L-GLU), strychnine hydrochloride, urethane, and UCN3. All of the solutions for the microinjections were freshly prepared in aCSF. The composition of aCSF (pH 7.4) was as follows (in mM): 128 NaCl, 3 KCl, 1.2 CaCl$_2$, 0.8 MgCl$_2$, 3.4 dextrose, and 5 HEPES. Where applicable, the concentration of drugs refers to their salts. The vendors for different drugs and chemicals were as follows: astressin 2B, d-AP7, and NBQX (R&D Systems, Minneapolis, MN), isoflurane (Baxter Pharmaceutical Products, Deerfield, IL), and UCN3 (American Peptide, Sunnyvale, CA). All other drugs and chemicals were obtained from Sigma Chemicals (St. Louis, MO).

Statistical analyses. Maximum changes in HR in response to microinjections of different drugs were expressed as means ± SE. One-way ANOVA followed by Tukey-Kramer’s multiple-comparison test was used for concentration-response study and determination of tachyphylaxis. Student’s paired t-test was used for comparison of the decreases in HR before and after microinjections of l-GLU and UCN3 into the nAmb. The differences were considered to be significant at $P < 0.05$.

RESULTS

Concentration-response of UCN3 into the nAmb. In this and other series of experiments, nAmb was always identified by unilateral microinjections of l-GLU (5 mM), which elicited bradycardia (83.6 ± 6 bpm) but no change in BP. Microinjections of different concentrations (0.031, 0.062, 0.125, 0.25, and 0.5 mM) of UCN3 into the nAmb elicited decreases in HR (5.3 ± 1.2, 9.5 ± 1.7, 13.7 ± 2.2, 19.2 ± 3.3, and 25.3 ± 4.3 bpm; Fig. 1). The maximal bradycardic responses were elicited by 0.25 mM concentration; therefore, this concentration of UCN3 was selected for further studies in other groups of rats. The onset, peak, and duration of the bradycardic responses elicited by 0.25 mM concentration of UCN3 were 5–15 s, 40–90 s, and 6–8 min, respectively (n = 15). In this and other experiments reported in this study, microinjections (30 nl) of aCSF did not elicit any cardiovascular responses.
Effect of intravenous administration of UCN3 on BP and HR. As mentioned earlier, microinjection of UCN3 (0.25 mM, 30 nl) into the nAmb, at 40-min intervals, elicited similar decreases in HR (45.8 ± 1, 40.5 ± 3.4, and 44.3 ± 1.4 bpm, respectively; P > 0.05; n = 5), indicating that repeated microinjections of UCN3 into the nAmb did not exhibit tachyphylaxis when the interval between microinjections was at least 40 min.

Site specificity of UCN3 responses. Microinjection of UCN3 (0.25 mM) into the adjacent regions of nAmb did not elicit any changes in BP and HR (n = 5). For example, microinjection of UCN3 into a site 0.48–0.64 mm rostral, 2.6–2.8 mm lateral to calamus scriptorius, and 2.5 to 2.8 mm deep from dorsal medulla did not show any changes in BP and HR; in this experiment, the baseline values for BP and HR were 95.6 ± 21.8 (mmHg) and 410.2 ± 4.5 (bpm), respectively, which did not change after the microinjection of UCN3 (0.25 mM).

Effect of vagotomy on UCN3-induced bradycardia. Bilateral vagotomy abolished the bradycardic responses elicited by microinjections of L-GLU, as well as UCN3 into the nAmb (Fig. 2). In this group of rats (n = 5), the decreases in HR induced by microinjections of L-GLU (5 mM) into the nAmb before and after the vagotomy were 80 ± 7.3 and 2.5 ± 1.1 bpm, respectively (P < 0.001). Similarly, the decreases in HR elicited by microinjections of UCN3 (0.25 mM) into the nAmb before and after the vagotomy were 46.7 ± 3.3 and 1.7 ± 1 bpm, respectively (P < 0.001).

Table 1. Effect of different CRF 2 receptor antagonists on UCN3-induced responses

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>UCN3-Induced Decreases in HR, bpm</th>
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<tbody>
<tr>
<td></td>
<td>Before</td>
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<tr>
<td>K41498, 0.125 mM</td>
<td>61.2 ± 5.9</td>
</tr>
<tr>
<td>K41498, 0.25 mM</td>
<td>51.1 ± 3.5</td>
</tr>
<tr>
<td>K41498, 0.5 mM</td>
<td>56.1 ± 4.5</td>
</tr>
<tr>
<td>K41498, 1 mM</td>
<td>55.6 ± 5.5</td>
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<tr>
<td>Astressin 2B, 0.125 mM</td>
<td>60 ± 7.6</td>
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<tr>
<td>Astressin 2B, 0.25 mM</td>
<td>48.3 ± 4.0</td>
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<tr>
<td>Astressin 2B, 0.5 mM</td>
<td>51.4 ± 3.9</td>
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</table>

The antagonists as well as UCN3 (0.25 mM) were microinjected into the nAmb, which was always identified by prior microinjections of L-GLU (5 mM). HR, heart rate; bpm, beats per minute; UCN3, urocortin 3. *P < 0.05; **P < 0.001; ***P < 0.0001; n = 6.
nAmb did not elicit any changes in HR; the values of HR before and after the microinjections of this antagonist were 401 ± 9.4 and 396.5 ± 9.1 bpm, respectively (P > 0.05).

**Effect of gabazine and strychnine on UCN3-induced bradycardia.** The nAmb was identified as usual using microinjections of L-GLU (5 mM). Five minutes later, UCN3 (0.25 mM) was microinjected into the nAmb, which elicited a decrease in HR (6.4 ± 0.6%). After an interval of 40 min, gabazine (0.01 mM) was microinjected into the nAmb; a decrease in baseline HR (16.3 ± 3.1 bpm) was elicited (n = 5). Two minutes later, UCN3 (0.25 mM) was again microinjected at the same site; the responses to UCN3 were blocked. F: responses to L-GLU (5 mM) were unaltered by K41498. PAP, pulsatile arterial pressure; MAP, mean arterial pressure; HR, heart rate.

UCN3 (0.25 mM) was microinjected at the same site, and a decrease in HR (8.2 ± 0.7%) was elicited. After an interval of 40 min, iGLUR antagonists D-AP7 (5 mM) and NBQX (2 mM) were microinjected into the nAmb; a small decrease in baseline HR (11.4 ± 1.7 bpm) was elicited. Within 2 min, UCN3 (0.25 mM) was microinjected again at the same site; UCN3-induced bradycardia (1.5 ± 0.2%) was significantly (P < 0.0001) attenuated. (Fig. 5C). The concentrations of D-AP7 and NBQX used in this experiment were selected from our previous reports (11, 27).

**Effect of blockade of ionotropic glutamate receptors (iGLURs) on UCN3-induced bradycardia.** Identification of the nAmb was done by microinjections of L-GLU (n = 5). Five minutes later, UCN3 (0.25 mM) was microinjected into the nAmb; a decrease in HR (8.2 ± 0.7%) was elicited. After an interval of 40 min, testisin 2B (a CRF2R antagonist; 0.25 mM) was microinjected at the same site; the antagonist elicited no significant responses. E: a 2-min interval was allowed for the diffusion of the antagonist into the nAmb and UCN3 (0.25 mM) was again microinjected at the same site; the responses to UCN3 were blocked. F: responses to L-GLU (5 mM) were unaltered by testisin 2B.

![Fig. 3. Tracing showing blockade of UCN3 responses in the nAmb by K41498.](image)

**Fig. 3.** Tracing showing blockade of UCN3 responses in the nAmb by K41498. A: bradycardic responses to microinjection of L-GLU (5 mM). B: after allowing an interval of 5 min, the vehicle (artificial cerebrospinal fluid, aCSF) was microinjected at the same site; no significant changes were observed. C: UCN3 (0.25 mM) was microinjected at the same site; bradycardic responses were elicited. D: after an interval of 40 min to avoid tachyphylaxis to UCN3 responses, K41498 (a CRF2 receptor antagonist; 0.5 mM) was microinjected at the same site; the antagonist elicited no significant responses. E: a 2-min interval was allowed for the diffusion of the antagonist into the nAmb and UCN3 (0.25 mM) was again microinjected at the same site; the responses to UCN3 were blocked. F: responses to L-GLU (5 mM) were unaltered by K41498. PAP, pulsatile arterial pressure; MAP, mean arterial pressure; HR, heart rate.

![Fig. 4. Tracing showing blockade of UCN3 responses in the nAmb by testisin 2B.](image)

**Fig. 4.** Tracing showing blockade of UCN3 responses in the nAmb by testisin 2B. A: bradycardic responses to microinjection of L-GLU (5 mM). B: after an interval of 5 min, the vehicle (aCSF) was microinjected at the same site; no significant changes were observed. C: UCN3 (0.25 mM) was microinjected at the same site; bradycardic responses were elicited. D: after an interval of 40 min, testisin 2B (a CRF2R antagonist; 0.25 mM) was microinjected at the same site; the antagonist elicited no significant responses. E: UCN3 (0.25 mM) was again microinjected at the same site; after a 2-min interval; the responses to UCN3 were blocked. F: responses to L-GLU (5 mM) were unaltered by testisin 2B.

![Fig. 5. The effect of blockade of GABA, glycine, and ionotropic glutamate receptors in the nAmb on UCN3 responses.](image)

**Fig. 5.** The effect of blockade of GABA, glycine, and ionotropic glutamate receptors in the nAmb on UCN3 responses. The bradycardic responses elicited by microinjections of UCN3 (0.25 mM) into the nAmb were significantly attenuated by microinjections of gabazine (0.01 mM) (A), strychnine (0.5 mM) (B), and combined microinjections of D-AP7 (5 mM) and NBQX (2 mM) into the nAmb (C). **P < 0.001. ***P < 0.0001.
Identification of immunoreactive cells containing CRF2Rs and UCN3. Immunohistochemical procedures (n = 6) showed the presence of cells containing CRF2Rs (Fig. 6A) and UCN3 (Fig. 6D) in the nAmb. Specificity of the primary antibody for CRF2Rs was indicated by the lack of staining for CRF2Rs when the primary antibody was either omitted (Fig. 6B) or preabsorbed by incubation with CRF2R control peptide (Fig. 6C). Similarly, specificity of the primary antibody for UCN3 was indicated by lack of staining for this peptide when the primary antibody was omitted (Fig. 6E) or preabsorbed by incubation with UCN3 control peptide (Fig. 6F).

Histological identification of microinjection sites. The microinjection sites of L-GLU (5 mM) and UCN3 (0.25 mM) in the nAmb were marked with a microinjection (30 nl) of green retrobeads (Lumafluor) (n = 30) (Fig. 7).

DISCUSSION

The present study showed that microinjections of UCN3 into the nAmb elicited bradycardia that was blocked by prior microinjections of selective CRF2R antagonists (K41498 and astressin 2B) (19, 40). The effects of K41498 and astressin 2B persisted for at least 60 min, indicating that the blocking effect of these CRF2R antagonists was long-lasting. This observation suggested that the bradycardic responses were mediated via CRF2Rs in the nAmb. In this context, it may be noted that UCN3 has been reported to act only through CRF2Rs, and it is considered to be the endogenous ligand for these receptors (15, 17, 21). There are very few studies in which the presence of CRF2Rs and UCN3 has been determined in the brain tissue. In one study, CRF2R mRNA expression was not detected in the nAmb (45). In another report, nAmb was not mentioned as one of the sites containing UCN3 immunoreactivity (21). Contrary to these reports, we observed scattered cells containing CRF2Rs and UCN3 in the nAmb in the present study; availability of good antibodies for CRF2Rs and UCN3 may have facilitated our immunohistochemical studies. Moreover, we have pharmacologically demonstrated the presence of CRF2Rs in the nAmb (Ref. 7 and present study). At the present time, it
is not clear under what physiological or pathological conditions endogenous UCN3 is released in the nAmb.

We have previously reported that microinjections of UCN1 into the nAmb elicited bradycardia, which was mediated via CRF1Rs (11). The presence of CRF1Rs in the nAmb has been reported in the literature (45). UCN1 acts on both CRF1Rs and CRF2Rs (15). However, it is unlikely that bradycardic responses to UCN1 in the nAmb were mediated via CRF2Rs because these responses were completely blocked by prior microinjections of a selective CRF1R antagonist (11). The bradycardic responses, including their onset, peak, and duration, elicited by UCN1 (11) and UCN3 (this paper) in the nAmb were comparable. No significant differences were observed between the bradycardic responses, including their onset, peak, and duration, elicited by UCN1 (11) and UCN3 (this paper) in the nAmb. For example, decreases in HR elicited by maximally effective concentration of UCN1 and UCN3 (0.25 mM) were 46.9 ± 1.7 and 45.7 ± 2.7 bpm, respectively (P > 0.05). Similarly, the onset, peak, and duration of the responses elicited by UCN1 and UCN3 (0.25 mM each) were comparable; the onset, peak, and duration of the UCN1-induced responses were 5–20 s, 40–80 s, and 4–10 min, respectively (unpublished observations), and those of UCN3-induced responses were 5–15 s, 40–90 s, and 6–8 min, respectively (this article). This observation is consistent with previous reports in which microinjections of UCN1 and UCN3 into the nucleus of the solitary tract (NTS) elicited similar cardiovascular responses in urethane-anesthetized rats (27, 28, 47).

CRF2Rs, like CRF1Rs, are coupled to the guanosine triphosphate-binding protein Gs and stimulate adenylate cyclase activity in most systems (1, 15, 50). Although this intracellular signaling pathway mediates most of the actions of CRF2R activation, other signaling pathways may also be involved (16). Activation of CRF2Rs by UCN3 and CRF1Rs by UCN1 may stimulate cardiovas- cular responses in the nAmb by an identical mechanism and elicit similar bradycardic responses.

Concentration-response studies using microinjections of UCN3 into the nAmb showed a nonlinear bell-shaped response. Several enzymes, peptides, and hormones show similar concentration responses. This type of concentration response has been explained by homotropic allosterly; in this model, higher concentrations of the peptide bind to a modulator site other than the primary binding site, modifying the function of the receptor, which results in attenuated responses (5).

Microinjections of aCSF into the nAmb did not elicit a response, indicating that local distortion of brain tissue was not responsible for the bradycardic effects elicited by microinjections of UCN3 at the same site. Leakage of UCN3, if any, from the microinjection site into the peripheral circulation was excluded because concentrations of UCN3 microinjections (0.25 mM, 30 nl) that elicited maximum decreases in HR did not elicit a response when injected intravenously. The site specificity of UCN3-induced bradycardic responses was indicated by the observation that identical microinjections of UCN3 into areas adjacent to the nAmb elicited no responses. On the basis of our experiments on unanesthetized decerebrate rats, we have previously reported that urethane anesthesia did not alter the responses to UCN3, qualitatively or quantitatively (28).

Because microinjections of the CRF2R antagonists alone into the nAmb did not elicit significant changes in basal HR, it was concluded that under normal physiological conditions, CRF2Rs in the nAmb were not under tonic control of endogenous UCN3. We have previously reported that UCNs may not be the neurotransmitters normally involved in mediating the cardiac responses to baroreflex activation (11). In this context, it may be noted that there is a general consensus that glutamate is the predominant neurotransmitter in the nAmb, mediating reflex bradycardic responses (47).

On the basis of our current knowledge regarding medullary control of cardiac function (14, 25, 41, 43, 47), the mechanism of the bradycardic responses elicited by UCN3 microinjections into the nAmb can be explained as follows. It is well established that cardiac vagal neurons in the nAmb are generally silent and depend on synaptic inputs for their activity (47). Major inputs to the cardiac vagal neurons in the nAmb include glutamatergic excitatory inputs and GABAergic and glyciner- gic inhibitory inputs. The glutamatergic inputs may arise from the NTS; in patch-clamp studies, electrical stimulation of the NTS activated glutamatergic currents in the cardiac vagal neurons in the nAmb via NMDA, AMPA, and kainate receptors (29). This NTS-nAmb pathway may play a role in baroreflex-induced vagally mediated reflex bradycardia (47). GABAergic inhibitory input also may arise from the NTS; stimulation of the NTS evokes GABAergic synaptic currents in the cardiac vagal neurons in the nAmb (47, 48). This GABAergic pathway from the NTS to the nAmb may mediate inhibition of cardiac vagal neurons during inspiration. Microinjections of glycine into the nAmb have been reported to increase HR, indicating the presence of glycine receptors in this nucleus (9). Anatomical studies have shown that cardiac vagal neurons are innervated by glycine-immunoreactive fibers (3) and receive glyciner- gic input (46,
The bradycardic responses elicited by microinjections of UCN3 were attenuated by microinjections of gabazine and strychnine, indicating that UCN3 inhibited GABAAergic and glycineric inputs to the cardiac vagal neurons, allowing predominance of the excitatory glutamatergic inputs to these neurons in the nAmb. Excitation of the cardiac vagal neurons in the nAmb by glutamatergic inputs is expected to increase vagal input to the heart and elicit bradycardia. This conclusion is supported by our observation that blockade of iGLURs in the nAmb by microinjections of d-AP7 and NBQX significantly attenuated the bradycardic responses elicited by UCN3. Excitation of neurons in the region surrounding the compact formation of the nAmb by UCN3 via CRF2Rs may have increased the activity of vagal input to the heart causing bradycardia. In this context, it may be noted that direct application of UCNs to neurons in other brain areas of the rat (e.g., cerebellar Purkinje neurons) has been reported to increase their firing rate (6). Recently, UCN3 has been reported to produce Ca$^{2+}$ influx and Ca$^{2+}$ release from internal stores in retrogradely labeled cardiovagal neurons in the nAmb; this cellular mechanism may be responsible for activation of these neurons (7).

Perspectives and Significance

Stress is one of the common risk factors in cardiovascular diseases. UCNs have been reported to be involved in the regulation of stress, anxiety states, food consumption, and social behaviors (32, 33). It is also known that UCNs may play a role in central cardiovascular regulation (11, 27, 28, 51). UCN3 may be released in the nAmb in yet unidentified pathological states (e.g., heart failure) and elicit bradycardia. This effect would counterbalance tachycardia induced by the disease. UCNs can reach the CNS via a unique transport system (33). Cardioprotective effects of UCNs have been reported in heart failure and ischemia/reperfusion injury (2, 36, 37, 38, 42). These beneficial effects of UCNs may be mediated via direct actions on myocardial tissue. For example, intravenous administration of UCNs elicits beneficial effects in experimental heart failure by increasing cardiac output and reducing peripheral resistance (2, 36, 37, 38, 42). In this situation, centrally induced bradycardia by UCN3 would be beneficial. In the present paper, we have described the mechanism by which UCN3 exerts bradycardic effects when microinjected into the nAmb. As mentioned in the introduction, the importance of nAmb in the central regulation of cardiac function is well established (4, 8, 24, 25, 30, 31, 44). The results of the present study provide a platform on which future studies can be designed for investigating the role of CRF2Rs in the nAmb in cardiac regulation during pathological states.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: V.C.C., K.K., and H.N.S. conception and design of research; V.C.C. and K.K. performed experiments; V.C.C., K.K., and H.N.S. analyzed data; V.C.C. and H.N.S. interpreted results of experiments; V.C.C. and K.K. prepared figures; V.C.C. and H.N.S. drafted manuscript; V.C.C., K.K., and H.N.S. approved final version of manuscript.

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