Depressor and bradycardic responses to microinjections of endomorphin-2 into the NTS are mediated via ionotropic glutamate receptors

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Kasamatsu, Ken, Vineet C. Chitravanshi, and Hreday N. Sapru. Depressor and bradycardic responses to microinjections of endomorphin-2 into the NTS are mediated via ionotropic glutamate receptors. Am J Physiol Regul Integr Comp Physiol 287: R715–R728, 2004; 10.1152/ajpregu.00642.2003.—The presence of endomorphin-like immunoreactivity has been reported in the nucleus tractus solitarius (NTS). It was hypothesized that endorphins may play a role in cardiovascular regulation in the medial subnucleus of the NTS (mNTS). Endomorphin-2 (E-2, 0.1–4 mmol/l) was microinjected (100 nl) into the mNTS of urethane-anesthetized, artificially ventilated, adult male Wistar rats. E-2 (0.2 mmol/l) elicited decreases in mean arterial pressure (40 ± 3.5 mmHg) and heart rate (50 ± 7.0 beats/min). These responses were blocked by prior microinjections of naloxonazine (1 mmol/l) into the mNTS. Responses to microinjections of E-2 into the mNTS were abolished by prior combined microinjections of d-2-amino-7-phosphonoheptanoic acid (an NMDA receptor antagonist, 5 mmol/l) and 2,3-dioxo-6-nitro-1,2,3,4-tetrahydro-7-pyridazino-7-sulfonamide disodium (a non-NMDA receptor antagonist, 2 mmol/l) into the mNTS. These results were confirmed by extracellular neuronal recordings. Blockade of GABAergic receptors in the mNTS by prior combined microinjections of gabazine (a GABA_A receptor antagonist, 2 mmol/l) and 2-hydroxyxsaclofen (a GABA_B receptor antagonist, 100 mmol/l) also blocked the responses to E-2. It was concluded that 1) the depressor and bradycardic responses to microinjections of E-2 into the mNTS are mediated via μ_1-opioid receptors as well as ionotropic glutamate receptors, 2) GABAergic neurons in the mNTS, which may inhibit the release of glutamate from nerve terminals, are inhibited by E-2 via μ_1-opioid receptors, and 3) disinhibition caused by the inhibition of GABAergic neurons by E-2 may result in an increase in the glutamate release from nerve terminals, which, in turn, may elicit depressor and bradycardic responses.

Bradycardia; depressor responses; opioid peptides

THE MEDIAL SUBNUCLEUS of the nucleus tractus solitarius (mNTS) is known to play an important role in the regulation of cardiovascular function (29, 35). Peripheral afferents (e.g., baroreceptor and cardiopulmonary afferents) make their primary synapse in this subnucleus (1), and microinjections of l-glutamate (l-Glu), which stimulates neuronal cell bodies but not passing fibers, into this subnucleus elicit depressor and bradycardic responses in anesthetized or unanesthetized decerebrate animals (5).

In recent years, three major subtypes of opioid receptors (μ, δ, and κ) have been identified (11, 18). Enkephalins and dynorphin A (1–16, 19) have been reported to be endogenous ligands for δ- and κ-opioid receptors, respectively (18). Recently, two tetrapeptides (endomorphin-1 and -2) were isolated from human cortex and bovine hypothalamus. The two peptides have been reported to possess a high affinity and selectivity for the μ-opioid receptors and are considered to be endogenous ligands for these receptors (11, 39). The presence of endomorphin-like immunoreactivity has been demonstrated in the mNTS (20, 25). On the basis of these reports, it was hypothesized that endorphins may play a role in the mediation and/or modulation of cardiovascular function in the mNTS. Except for one preliminary report from this laboratory (30), there is no report in the literature in which the role of endorphins in the cardiovascular regulation at the level of mNTS has been studied.

MATERIALS AND METHODS

General procedures. Adult male Wistar rats (Charles River Laboratories, Wilmington, MA; 300–350 g body wt, n = 122) were housed under controlled conditions with a 12:12-h light-dark cycle. Food and water were available to the animals ad libitum. The experimental procedures were performed in accordance with the American Physiological Society guidelines for animal experimentation, and the Institutional Animal Care and Use Committee of this university approved the experimental protocols. The minimum number of animals required for statistical analyses of the data was used, and every effort was made to minimize any suffering to the animals.

Anesthesia was induced by administration of isoflurane (3% in 100% oxygen) via a nose mask. The trachea was cannulated with polyethylene (PE-240) tubing, the rats were artificially ventilated using a rodent ventilator (model 683, Harvard Instruments, Holliston, MA), and anesthesia was maintained by tracheal administration of isoflurane (2–3% in 100% oxygen). The femoral vein and artery on one side were cannulated with polyethylene (PE-50) tubing for intravenous injections of various agents and for monitoring blood pressure (BP), respectively. A pressure transducer (model P23 Db, Grass Instruments, West Warwick, RI) was used to measure BP. Heart rate (HR) was monitored by a tachograph (model 7P4, Grass Instruments) that was triggered by the BP waves. Urethane (1.2–1.4 g/kg) was injected intravenously in six or seven aliquots at 2-min intervals. The tracheal inflation of isoflurane was discontinued after administration of the third aliquot of urethane. The depth of anesthesia was established by pinching the hindpaw of the rat; absence of a BP response and/or withdrawal of the limb indicated that the rat was properly anesthetized. Rectal temperature was monitored continuously and maintained at 37 ± 0.5°C using an infrared lamp connected to a temperature controller. All the traces were recorded on a polygraph (model 7D, Grass Instruments).

To determine the role of parasympathetic innervation to the heart in mediating the HR responses elicited by microinjections of endomorphin-2 (E-2) into the mNTS, silk sutures were placed loosely around the vagus nerves bilaterally for subsequent identification and sectioning of the nerves.

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Microinjection technique. The rats were placed in a prone position in a stereotaxic instrument (model 1430, David Kopf Instruments, Tujunga, CA) with the bite bar 18 mm below the interaural line. The medulla was exposed by removal of the dorsal neck muscles and parts of the occipital bone and dura mater. The atlantooccipital membrane was incised. Four-barreled glass micropipettes (tip size 20–40 μm) were mounted on a micromanipulator (model 1460, David Kopf Instruments), and each barrel was connected via polyethylene tubing to one of the channels of a picospritzer (General Valve, Fairfield, NJ). One of the barrels contained L-Glu, and the contents of the other barrels varied according to the requirements of the experiment. The coordinates for the mNTS were as follows: 0.5–0.6 mm rostral and 0.5–0.6 mm lateral to the calamus scriptorius and 0.5–0.6 mm deep from the dorsal medullary surface. For making microinjections, the micropipette was inserted into the brain perpendicularly. The site eliciting depressor and bradycardic responses was identified by microinjections of L-Glu (5 mmol/l) into the mNTS. The volume of microinjections (100 nl) was visually confirmed by displacement of the fluid meniscus in the barrel containing the injectate under a modified binocular horizontal microscope with a graduated reticule in one eyepiece (model PZMH, World Precision Instruments, Sarasota, FL). The duration of the microinjection was 10 s. Controls for microinjections consisted of artificial cerebrospinal fluid (aCSF; see Drugs and chemicals).

Extracellular neuronal recording. A seven-barreled glass micropipette (Medical Systems, Greenvale, NY) was pulled in a vertical pipette puller (model PE-2, Narishige, Tokyo, Japan). The tip size was adjusted so that the resistance of the barrels was 4–8 MΩ. The resistance of the micropipette barrels was measured at 1.000 Hz in saline by a microelectrode tester (model BL-1000, Winston Electronics, San Francisco, CA). The micropipette was mounted on a micromanipulator (model 1460, David Kopf Instruments, with an AP slide model 1262). The barrel used for recording was filled with 4 M NaCl, the second barrel contained aCSF, which was ejected on neurons as a control solution, the third barrel contained diluted India ink for marking the site of recording, and the other barrels were filled with agonists and antagonists. The mNTS site was identified by elicitation of depressor and bradycardic responses to microinjections of L-Glu (5 mmol/l, 100 nl) via a single-barreled micropipette (tip size 20–40 μm). After the coordinates were noted, the single-barreled micropipette was withdrawn, and a seven-barreled micropipette, used for recording and ejection of different agents, was inserted into the mNTS using these coordinates. It was not necessary to insert the recording micropipette in the same track that was used for microinjections of L-Glu; the latter was used as a guide for the general area of the mNTS involved in cardiovascular regulation. The involvement of recorded neurons in cardiovascular regulation was ascertained by the responses of the neuron to increases in systemic BP elicited by injections of phenylephrine (3 μg/kg iv; see RESULTS). The recording barrel was connected to a probe head stage, and the spontaneous action potentials were amplified using an amplifier (model DAM 80, World Precision Instruments, with filters set at 300–10,000 Hz), fed into a window discriminator (model 2503, Frederick Haer, Brunswick, ME), visualized on an oscilloscope (model R5103N, Tektronix, Beaverton, OR), digitized by an analog-to-digital converter (model DR-890, Neurodata Instruments, Delaware Gap, PA), and recorded on a video cassette recorder (model HR-A591U, JVC, VHS 4-head). The transistor-transistorlogic pulses generated from the window discriminator were fed into a rate/interval counter (model RIC-830, CWE, Ardmore, PA) to monitor the firing rate of the neurons (spikes/s). The amplitude of neuronal activity (μV) was assessed from the signal on the oscilloscope screen and the settings on the amplifiers. Ejection of contents in different barrels on neurons was accomplished by application of pressure pulses (1.5-ms duration, 40 pounds/in² gauge), and the volume of ejection solution (5 nl) was visually confirmed under a modified binocular horizontal microscope (see Microinjection technique).

Histology. The accuracy of our placement of microinjections into the mNTS where L-Glu elicited baroreceptor reflex-like responses has been repeatedly demonstrated by histological examination in our previous publications (5). In this study, typical sites of microinjections or neuronal recordings were marked by a unilateral microinjection (10–50 nl) of diluted India ink. The animals were perfused with arterial administration of heparinized normal saline followed by 10% formalin, and the brains were removed and fixed in 10% formalin for 24 h. After the fixation procedure was completed, serial sections were cut (30 μm) in a cryostat and mounted on slides. The sections were then stained with cresyl violet, and the microinjection or recording site (marked with India ink) was identified under a microscope (model AX70, Olympus Provis, Middlebush, NJ). The sections were photographed and compared with a standard atlas (23).

Statistical analyses. Means ± SE were calculated for maximum decreases in mean arterial pressure (MAP) and HR in response to microinjections of E-2 or L-Glu. Comparisons of decreases in MAP elicited by different concentrations of the agonists, were made by one-way analysis of variance followed by Tukey-Kramer multiple comparison test. Comparisons of the maximum changes in MAP and HR elicited by E-2 before and after the microinjections of different receptor antagonists were made by paired t-test. In all cases, the differences were considered significant at P < 0.05.

Drugs and chemicals. The following drugs and chemicals were used, E-2 (a μ-receptor agonist) (11, 39), naloxonazine hydrochloride (a μ1-receptor antagonist) (26), d-2-amino-7-phosphonohexanoic acid (D-AP7, an NMDA receptor antagonist) (5), L-glutamate monosodium, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzof[l]quinoline-7-sulfonamide disodium (NBQX disodium salt, a non-NMDA receptor antagonist) (5), muscimol hydrobromide (a GABA A receptor agonist) (33), baclofen hydrochloride (a GABA B receptor antagonist) (33), gabazine bromide (a GABA A receptor antagonist) (13), 2-hydroxy-saclofen (a GABA B receptor antagonist), isoflurane, and urethane. All solutions for the microinjections were freshly prepared in aCSF. The composition of aCSF (pH 7.4) was as follows (in mmol/l): 128 NaCl, 3 KCl, 1.2 CaCl2, 0.8 MgCl2, 3.4 dextrose, and 5 HEPES (5). The control solutions for microinjections and neuronal recordings consisted of 100 and 5 nl of aCSF, respectively. Where applicable, the concentration of drugs injected into the mNTS refers to their salts. All drugs were obtained from Sigma Chemical (St. Louis, MO). Isoflurane was purchased from Baxter Pharmaceutical Products (Deerfield, IL.).

RESULTS

Concentration response of E-2. In this and other series of experiments, the mNTS was always identified by microinjections of L-Glu (5 mmol/l), which stimulates neurons but not fibers of passage. To select the appropriate volume of microinjections, E-2 (0.2 mmol/l) was microinjected into the same site of the mNTS in 100- and 50-nl volumes (n = 4). The decreases in MAP were 36 ± 1.8 and 22 ± 1.2 mmHg for 100- and 50-nl volumes, respectively (P < 0.05). Similarly, the decreases in HR were 41 ± 4.5 and 22 ± 2.0 beats/min for 100- and 50-nl volumes, respectively (P < 0.05). Because the responses were greater when the volume of microinjection was 100 nl, this volume was selected for all microinjections. Concentration response for E-2 was studied in the mNTS (0.5–0.6 mm rostral to the calamus scriptorius, 0.5–0.6 mm lateral to the midline, and 0.5–0.6 mm deep from the dorsal medullary surface) where microinjections of L-Glu induced depressor (41 ± 1.5 mmHg) and bradycardic (38 ± 3.3 beats/min) responses. The interval between the microinjections of L-Glu and other agents was ≥5 min. Microinjections of E-2 (0.1, 0.2, 0.5, 1, 2, and 4 mmol/l) into the mNTS (n = 30, 5 rats for each concentration) elicited decreases in MAP (25 ± 5.3, 40 ± 2.8,
responses to microinjections of 0.2 mmol/l E-2 were elicited by 0.2 mmol/l E-2. The onset and durations of the responses to microinjections of 0.2 mmol/l E-2 were 5–10 s and 8–10 min, respectively, and the peak effect was observed at 2–3 min. The depressor responses to higher concentrations (1, 2, and 4 mmol/l) were significantly smaller ($P < 0.05$) than those elicited by 0.2 mmol/l E-2. In these experiments, only one concentration was microinjected into the mNTS of each animal. For comparison of these results, a cumulative dosing approach was used in another group of rats ($n = 5$). A site where depressor and bradycardic responses were elicited by microinjections of L-Glu was identified. Then 0.1, 0.2, and 0.5 mmol/l E-2 were microinjected at the same site in random order. The interval between different microinjections was 20 min. The decreases in MAP in response to 0.1, 0.2, and 0.5 mmol/l E-2 were 24.1 ± 2.7, 37.5 ± 2.8, and 30 ± 3.1 mmHg, respectively. The decreases in HR in response to the same concentrations of E-2 were 20 ± 3.1, 47.5 ± 6.2, and 32.5 ± 4.7 beats/min, respectively. Maximal depressor and bradycardic responses were elicited by 0.2 mmol/l E-2. Comparison of the responses in which only one concentration was injected in each animal and with those in which a cumulative dosing approach was used showed that the differences in the responses to each concentration (i.e., 0.1, 0.2, and 0.5 mmol/l) were not statistically different ($P > 0.05$). When the concentrations of E-2 that elicited depressor and bradycardic responses in the mNTS (e.g., 0.2 mmol/l) were injected intravenously, no responses were elicited.

In another group of rats ($n = 5$), the concentration of E-2 that elicited maximal cardiovascular responses was microinjected into the mNTS at least three times at 20-min intervals. The decreases in MAP in response to three consecutive microinjections of E-2 (0.2 mmol/l) were 32.5 ± 3.3, 33.3 ± 2.5, and 32.5 ± 3.4 mmHg, respectively. The decreases in HR in response to these microinjections of E-2 were 32.5 ± 3.1, 36.7 ± 4.2, and 28.3 ± 4.0 beats/min, respectively. Thus no tachyphylaxis of responses was observed with repeated microinjections of E-2.

The possibility that the cardiovascular responses elicited by microinjections (100 nl) of E-2 into the mNTS may have resulted from the spread of the injectate to adjacent regions was tested in another group of rats ($n = 5$). In these experiments, a site in the mNTS where microinjections of L-Glu elicited depressor and bradycardic responses was first identified in each rat. Microinjections (100 nl) of E-2 (0.2 mmol/l) at the same site elicited decreases in MAP (36 ± 2.5 mmHg) and HR (42 ± 3.7 beats/min). In the same rat, L-Glu (5 mmol/l) and E-2

![Fig. 1](http://ajpregu.physiology.org/)

Fig. 1. A: concentration response for mean arterial pressure (MAP). Microinjections of endomorphin-2 (E-2) at 0.1, 0.2, 0.5, 1, 2, and 4 mmol/l into the medial subnucleus of the nucleus tractus solitarius (mNTS, $n = 5$ for each concentration) elicited decreases in MAP: 25 ± 5.3, 40 ± 2.8, 30 ± 3.1, 16.6 ± 4.5, 18 ± 3.4, and 15 ± 1.5 mmHg, respectively. B: concentration response for heart rate (HR). Microinjections of E-2 at 0.1, 0.2, 0.5, 1, 2, and 4 mmol/l into the mNTS elicited decreases in HR: 20 ± 3.2, 50 ± 7.0, 37.5 ± 10.3, 33.3 ± 8.8, 20 ± 10.0, and 15 ± 2.9 beats/min, respectively. C: decrease in MAP elicited by E-2 before and after (28.7 ± 2.4 and 26.3 ± 1.3 mmHg, respectively) ipsilateral vagotomy was not statistically different ($P > 0.05$). D: decrease in HR elicited by E-2 before and after (42.5 ± 4.8 and 23.8 ± 3.8 beats/min, respectively) ipsilateral vagotomy (Vag-X) was significant ($P < 0.05$). E: decrease in MAP elicited by E-2 before and after (27 ± 2.5 and 21.3 ± 1.3 mmHg, respectively) bilateral vagotomy was not statistically different ($P > 0.05$). F: decrease in HR elicited by E-2 before bilateral vagotomy (40 ± 9.1 beats/min) was completely abolished after bilateral vagotomy. A period of 15 min was allowed after vagotomy for stabilization. *Significantly different from before ipsilateral or bilateral vagotomy.
(0.2 mmol/l) were microinjected (100 nl each) into an adjacent area (0.5 mm rostral to the calamus scriptorius, 1.2 mm lateral to the midline, and 0.5 mm deep from the dorsal medullary surface). This region included the cuneate nucleus. Microinjections of l-Glu and E-2 into this site elicited no cardiovascular responses. In the same rat, similar microinjections of l-Glu and E-2 into the ventrolateral NTS (0.5–0.6 mm rostral to the calamus scriptorius, 1.2–1.4 mm lateral to the midline, and 1.1–1.2 mm deep from the dorsal medullary surface) elicited no cardiovascular responses.

E-2-induced bradycardia is vagally mediated. In one group of rats (n = 4), the decreases in MAP before and after ipsilateral vagotomy were 28.7 ± 2.4 and 26.3 ± 1.3 mmHg, respectively; thus ipsilateral vagotomy did not elicit a significant effect (P > 0.05) on E-2-induced depressor responses (Fig. 1C). In this group of rats, the decreases in HR before and after ipsilateral vagotomy were 42.5 ± 4.8 and 23.8 ± 3.8 beats/min, respectively; thus ipsilateral vagotomy elicited a significant attenuation (P < 0.05) of E-2-induced bradycardia (Fig. 1D). A stabilization period of 15 min was allowed after ipsilateral or bilateral vagotomy. In another group of rats (n = 4), the decreases in MAP before and after bilateral vagotomy were 27.5 ± 2.5 and 21.3 ± 1.3 mmHg, respectively; thus bilateral vagotomy did not alter significantly (P > 0.05) the depressor responses to E-2 (Fig. 1E). In the same group of rats, the E-2-induced decrease in HR was 40 ± 9.1 beats/min, which was completely abolished by bilateral vagotomy (Fig. 1F).

μ1-Opioid receptors mediate E-2-induced responses. The mNTS region from which depressor and bradycardic responses were elicited was identified by a microinjection of l-Glu (5 mmol/l, n = 5; Fig. 2A). Within 5 min, microinjections of aCSF (100 nl) at the same site elicited no responses (Fig. 2B). After 2 min, microinjection of E-2 (0.2 mmol/l) at the same site elicited decreases in MAP and HR (Fig. 2C). At 20 min after recovery of the responses, naloxonazine (1 mmol/l) was microinjected at the same site. Microinjection of naloxonazine (1

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**Fig. 2.** Blockade of E-2 responses by naloxonazine. A: mNTS was identified by a microinjection of l-Glu (5 mmol/l). B: microinjection of artificial cerebrospinal fluid (aCSF) 5 min after l-Glu did not elicit a response and did not alter response to subsequent injection of E-2. C: microinjection of E-2 (0.2 mmol/l) 2 min after aCSF elicited depressor and bradycardic responses. D: microinjection of naloxonazine (1 mmol/l) alone 20 min after recovery of E-2-induced responses did not elicit a response. E: E-2 microinjected 2 min after naloxonazine failed to elicit a response. F: l-Glu microinjected 2 min after E-2 continued to elicit depressor and bradycardic responses. G and H: abolition of depressor and bradycardic responses to microinjections of E-2 by naloxonazine (Naloxo); decreases in MAP and HR before microinjection of naloxonazine were 36 ± 2.4 mmHg and 20 ± 4.5 beats/min, respectively. These responses were abolished (+) after naloxonazine. Arrows indicate microinjections into mNTS.

PAP, pulsatile arterial pressure.
mmol/l) alone elicited no cardiovascular responses (Fig. 2D). At 2 min after the microinjection of naloxonazine, microinjection of E-2 (0.2 mmol/l) failed to elicit any response (Fig. 2E). The blocking effect of naloxonazine lasted for 15e20 min. The lack of responses to E-2 was not due to tachyphylaxis, because repeated microinjections of E-2 did not result in tachyphylaxis, as described earlier. This concentration of naloxonazine (1 mmol/l) was selected for blockade of E-2 responses, because other experiments (n = 4) showed that a smaller concentration (0.5 mmol/l) did not significantly (P > 0.05) block the responses to E-2 (0.2 mmol/l). The specificity of naloxonazine (1 mmol/l) was indicated by the observation that this antagonist did not alter the depressor and bradycardic responses to L-Glu (5 mmol/l) microinjected 2 min after E-2 (Fig. 2F). Group data for the effects of naloxonazine on E-2 responses are presented in Fig. 2, G and H. The E-2 (0.2 mmol/l)-induced decreases in MAP and HR before the microinjection of naloxonazine were 36 ± 2.4 mmHg and 20 ± 4.5 beats/min, respectively. Naloxonazine completely abolished these responses. Group data for the effects of naloxonazine on t-Glu responses (not shown in Fig. 2) indicated that naloxonazine did not alter the responses to t-Glu; the decreases in MAP in response to microinjections of t-Glu (5 mmol/l) before and after microinjection of naloxonazine (1 mmol/l) were 44 ± 4.8 and 38 ± 3.4 mmHg, respectively (P > 0.05). Similarly, the decreases in HR in response to the same concentrations of t-Glu before and after microinjection of naloxonazine were 54 ± 14.5 and 46 ± 12.5 beats/min, respectively (P > 0.05).

**GABA-receptor blockade abolishes E-2-induced responses.** Microinjections of a GABA_A receptor agonist (muscimol, 0.025, 0.05, and 0.1 mmol/l) into the mNTS elicited pressor and tachycardic responses (n = 5; Table 1). Similar studies showed that microinjections of a GABA_B receptor agonist (baclofen, 0.05, 0.1, 0.5, and 1 mmol/l) into the mNTS elicited increases in MAP and HR (n = 5; Table 1). The minimum concentration of gabazine (a GABA_A receptor antagonist) that completely blocked the effect of the maximally effective concentration of muscimol (0.05 mmol/l) was 2 mmol/l. Similarly, the minimum concentration of 2-hydroxyasaclofen (a GABA_B receptor antagonist) that completely blocked the effects of the maximally effective concentration of baclofen (0.1 mmol/l) was 100 mmol/l. Traces of the effect of GABA receptor blockade on E-2 responses are shown in Fig. 3. The mNTS site was first identified by microinjections of L-Glu (Fig. 3A). Within 5 min, aCSF (100 nl) was microinjected at the same site, and no cardiovascular effects were elicited (Fig. 3B). After 2 min, E-2 (0.2 mmol/l) was microinjected at the same site, and decreases in MAP and HR were elicited (Fig. 3C). At 20 min after the recovery of responses, gabazine (2 mmol/l), followed 2 min later by 2-hydroxyasaclofen (100 mmol/l), was microinjected into the same mNTS site. Blockade of GABA receptors in the mNTS by combined microinjections of gabazine and 2-hydroxyasaclofen elicited 1- to 2-min decreases in MAP and >60-min decreases in HR (Fig. 3D). E-2 (0.2 mmol/l) was again microinjected at the same site within 2 min of the injection of 2-hydroxyasaclofen, and the decreases in MAP and HR were abolished by GABA receptor blockade (Fig. 3E). Gabazine and hydroxyasaclofen did not alter depressor responses to microinjections of L-Glu (Fig. 3F). Group data (n = 7) for the effect of gabazine and hydroxyasaclofen on E-2 responses are shown in Fig. 3, G and H. The decreases in MAP and HR elicited by E-2 (0.2 mmol/l) before the microinjections of gabazine and hydroxyasaclofen were 32.8 ± 2.8 mmHg and 40 ± 6.9 beats/min, respectively. At 2 min after the combined microinjections of gabazine and hydroxyasaclofen, the depressor and bradycardic responses to E-2 were abolished. The depressor responses to microinjections of L-Glu into the mNTS were not significantly attenuated; the decreases in MAP in response to L-Glu before and after the microinjections of gabazine and 2-hydroxyasaclofen were 37.5 ± 4.6 and 29.2 ± 3.0 mmHg, respectively (P > 0.05; group data not shown in Fig. 3). However, the L-Glu-induced bradycardia was attenuated by prior injections of gabazine and 2-hydroxyasaclofen; the decreases in HR in response to L-Glu before and after the microinjections of gabazine and 2-hydroxyasaclofen were 43.3 ± 6.1 and 25 ± 8.5 beats/min, respectively (P < 0.05). As stated earlier, microinjections of gabazine and 2-hydroxyasaclofen caused a significant decrease in baseline HR (50 ± 9.5 beats/min) that lasted for >60 min. Because of the long duration of the effects of GABA receptor blockade on HR responses, it was not possible to interpret the lack of bradycardic responses to microinjections of E-2 and L-Glu after the microinjections of gabazine and 2-hydroxyasaclofen.

**Blockade of ionotropic glutamate receptors abolishes E-2-induced responses.** Traces in one animal showing the effects of ionotropic glutamate receptors in the mNTS on E-2-induced responses are presented in Fig. 4. The mNTS was identified by microinjection of carbachol (0.5 mmol/l; Fig. 4A). Microinjection of aCSF (100 nl) at the same site within 5 min elicited no response (Fig. 4B). Microinjection of E-2 (0.2 mmol/l) at the same site, 2 min after aCSF, elicited depressor and bradycardic responses (Fig. 4C). At 20 min after the recovery of responses, NBQX (2 mmol/l) and D-AP7 (5 mmol/l) were injected sequentially, 2 min apart, at the same site (Fig. 4D). After a 2-min period was allowed for the antagonists to diffuse into the mNTS, E-2 (0.2 mmol/l) was microinjected again into the same site. E-2 failed to elicit any response (Fig. 4E). Combined microinjections of NBQX and D-AP7 did not alter the depressor and bradycardic responses to an unrelated agonist, carbachol (0.5 mmol/l; Fig. 4F). Group data for these experiments are shown in Fig. 5. In one group of rats (n = 5), microinjections of E-2 (0.2 mmol/l) into the mNTS elicited decreases in MAP (40 ± 4.7 mmHg) and HR (20 ± 5.5 beats/min). These effects of E-2 were significantly

**Table 1. Cardiovascular effects of microinjections of GABA receptor agonists into the mNTS**

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<tr>
<th>Concen, mmol/l</th>
<th>Increase in MAP, mmHg</th>
<th>Increase in HR, beats/min</th>
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<tr>
<td><strong>Muscimol</strong></td>
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<td>0.025</td>
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Values are means ± SE; n = 5. Muscimol is a GABA_A receptor agonist, and baclofen is a GABA_B receptor agonist. MAP, mean arterial pressure; HR, heart rate; mNTS, medial subnucleus of nucleus tractus solitarius.
(P < 0.01) attenuated (MAP = 15 ± 3.9 mmHg, HR = 6 ± 6 beats/min) after the microinjection of D-AP7 (an NMDA receptor antagonist, 5 mmol/l) at the same site (Fig. 5, A and B).

In another group of rats (n = 5), NBQX (2 mmol/l, a non-NMDA receptor antagonist) also significantly attenuated (P < 0.01) the cardiovascular effects of E-2; the decreases in MAP in response to microinjections of E-2 before and after the microinjections of NBQX were 30 ± 1.5 and 12 ± 2.6 mmHg, respectively, and decreases in HR were 24 ± 4.8 and 8 ± 4.2 beats/min, respectively (Fig. 5, C and D). In a third group of rats (n = 5), non-NMDA and NMDA receptors were blocked by sequential microinjections of NBQX and D-AP7, 2 min apart. At 2 min after the microinjections of NBQX and D-AP7, E-2 microinjections (0.2 mmol/l) failed to elicit the depressor and bradycardic responses. For example, the decreases in MAP and HR in response to E-2 before the blockade of ionotrophic glutamate receptors were 34 ± 4.8 mmHg and 20 ± 7.7 beats/min, respectively. These responses were almost completely blocked by NBQX and D-AP7 (Fig. 5, E and F).

NBQX and D-AP7 did not alter responses to an unrelated agonist (carbachol). Group data for these experiments (n = 5) are summarized as follows. The decreases in MAP elicited by microinjections of carbachol (0.5 mmol/l, 100 nl) into the mNTS before and after the combined microinjections of NBQX and D-AP7 were 38 ± 6.5 and 40 ± 7.3 mmHg, respectively. Similarly, the decreases in HR elicited by microinjections of carbachol (0.5 mmol/l, 100 nl) into the mNTS remained unchanged after the combined microinjections of NBQX and D-AP7 (27.5 ± 4.7 beats/min; Fig. 5, G and H).

Neuronal recording. The results obtained in microinjection studies were confirmed by single-unit recordings as follows. In one group of rats (n = 7), the basal firing rate of mNTS neurons (10 neurons) was 15.9 ± 2.2 spikes/s (Figs. 6A and 7A). A bolus injection of phenylephrine (3 μg/kg iv) increased MAP (40 ± 4.8 mmHg) and increased neuronal firing in the
mNTS; the peak increase of 31.2 ± 6.8 spikes/s was reached within 3 s (P < 0.05); the firing of the neuron recovered to basal level within 5–6 s (Figs. 6B and 7B). Excitation of the neuron by an intravenous bolus injection of phenylephrine was considered to be an indication that the neuron is involved in baroreflex and, therefore, cardiovascular regulation. When BP returned to basal level, L-Glu (5 mmol/l) was applied to the neurons. The ejection volume of all direct applications of different agents on neurons was 5 nl. Application of L-Glu increased the neuronal firing to a maximum of 65.8 ± 14.2 (range 13–180) spikes/s (P < 0.01); the maximum increase was reached within 0.2 s, and the firing returned to basal level after 0.8 ± 0.1 s (Figs. 6C and 7C). Application of aCSF did not elicit a response, indicating that pressure applications alone were not responsible for the changes in neuronal firing (Figs. 6D and 7D). E-2 (0.2 mmol/l) was applied to the neurons 2–3 s after the aCSF. E-2 increased the firing of the mNTS neurons to a maximum of 31.6 ± 4.9 (range 14–58) spikes/s (P < 0.05); maximum firing was reached within 0.1–0.2 s, and the firing returned to basal level within 0.9 ± 0.1 s (Figs. 6E and 7E). Naloxonazine (1 mmol/l) was applied to the neuron 2–3 s after the application of E-2; no change in the neuronal firing compared with the basal firing rate (P > 0.05) was observed (Figs. 6F and 7F). Within 2–3 s, E-2 was again applied to the neurons. Naloxonazine blocked the excitatory effect of E-2; E-2-induced maximum firing rates of mNTS neurons before and after the application of naloxonazine were 31.6 ± 4.9 (range 14–58) and 10.9 ± 2.2 spikes/s, respectively (P < 0.05; cf. Figs. 6, E and G, and Fig. 7, E and G). After 1–3 min, application of E-2 (0.2 mmol/l) to the neurons again elicited an increase in the firing rate [24.2 ± 3.2 (range 9–42) spikes/s], indicating 76.5% recovery of the responses after the naloxonazine-induced blockade (Fig. 6H and 7H). Naloxonazine did not alter the responses to direct application of L-Glu (5 mmol/l); L-Glu-induced maximum firing rates of mNTS neurons before and after the application of naloxonazine were 65.8 ± 14.2 and 62.5 ± 11.3 spikes/s (P > 0.05; not shown in Figs. 6 and 7).

In another group of rats (n = 7), the basal rate of firing of mNTS neurons (24 neurons) was 8 ± 1.2 (range 3–60) spikes/s (Figs. 8A and 9A). Bolus injection of phenylephrine (3 μg/kg iv) increased the MAP (38 ± 4 mmHg) and neuronal firing to a maximum of 39.2 ± 6.6 spikes/s within 2 s (P < 0.05; Figs. 8B and 9B). Direct application of L-Glu (5 mmol/l) increased the neuronal firing to a maximum of 62.3 ± 16.5 (range 18–220) spikes/s (P < 0.05); the maximum increase in firing was reached within 0.1 s, and the neuronal firing returned to basal level within 0.4 ± 0.2 to 0.8 ± 0.2 s (Figs. 8C and 9C). Application of aCSF within 2–3 s did not alter the basal firing rate of the mNTS neurons (Figs. 8D and 9D). Direct application of E-2 (0.2 mmol/l) 2–3 s after aCSF increased the neuronal firing to a maximum of 44.6 ± 8.9 (range 12–120) spikes/s; the firing rate reached a maximum in 0.1 s and returned to basal level within 0.4 s (Figs. 8E and 9E). After 2–3 s, combined application of D-AP7 (5 mmol/l) and NBQX (2 mmol/l), with 1–3 s between the two injections, decreased the basal firing rate; the firing rate decreased to a maximum of 2 ± 1 spikes/s within 10–15 s (Figs. 8F and 9F). After the firing returned to basal level (40–50 s), subsequent application of E-2 (0.2 mmol/l) failed to excite the neuron; the E-2-induced increase in firing rate before the application of D-AP7 and NBQX was 44.6 ± 8.9 (range 12–120) spikes/s; after application of these agents, firing remained at basal levels (8 ± 1.2 spikes/s; cf. Figs. 8, E and G, and Fig. 9, E and G). After 1–3 min, application of E-2 (0.2 mmol/l) again increased the firing.
35.2 ± 6.5 (range 14–85) spikes/s; the increase in firing reached a maximum within 0.1–0.2 s. Thus there was a 77.8% recovery of the neuronal responses after the blockade of ionotropic glutamate receptors (Figs. 8H and 9H).

In another group of rats (n = 5), the basal rate of firing of mNTS neurons (15 neurons) was 18.9 ± 4.1 spikes/s (Fig. 10, A and G). Bolus injection of phenylephrine (3 μg/kg iv) increased the MAP to 40 ± 10 mmHg and neuronal firing to a maximum of 38.9 ± 4.5 spikes/s within 2 s (P < 0.01; Fig. 10, B and H). After 5–6 s, application of aCSF did not alter the basal firing rate of the mNTS neurons (Fig. 10, C and I). Direct application of carbachol (0.5 mmol/l) 2–3 s after aCSF increased the neuronal firing to a maximum of 45.2 ± 6.5 spikes/s within 0.2 s; the neuronal firing returned to basal level within 0.8 ± 0.2 s (Fig. 10, D and J). Combined application of D-AP7 (5 mmol/l) and NBQX (2 mmol/l) 2–3 s after carbachol decreased the basal firing rate to a maximum of 2 ± 1 spikes/s within 10–15 s and returned to basal level within 40–50 s (Fig. 10, E and K). Application of carbachol (0.5 mmol/l) 2–3 s after applications of D-AP7 and NBQX continued to elicit excitation of the neuron; the firing rate of mNTS neurons was 43.9 ± 6.4 spikes/s (cf. Fig. 10, D and F and Fig. 10, J and L; P > 0.05). Thus D-AP7 and NBQX did not alter significantly the effects of carbachol on mNTS neurons.

**Histology.** The mNTS sites where E-2 elicited excitatory effects by microinjections or direct neuronal applications were marked in 30 rats. A typical mNTS site marked with India ink (10 nl) is shown in Fig. 11A. This section represents a site for neuronal recording. In microinjection studies, a larger volume of India ink (50 nl) was injected at the sites from which responses were elicited. Composite diagrams of these sites are shown in Fig. 11, B and C, in which each spot represents a site of microinjection. Of the 30 spots, only 21 spots are visible in Fig. 11, A–C, because of overlapping. The sites marked with ink were located in the mNTS 0.5–0.6 mm rostral to the calamus scriptorius, 0.5–0.6 mm lateral to the midline, and 0.5–0.6 mm deep from the dorsal medullary surface.

**DISCUSSION**

The main finding of this study was that microinjections of E-2 (0.1–4 mmol/l) into the mNTS of the rat elicited depressor and bradycardic responses that were mediated via ionotropic glutamate receptors. Local distortion of brain tissue or any nonspecific effects were not responsible for these observations, because microinjections of aCSF into the mNTS did not elicit any response. Concentrations of E-2 microinjections into the mNTS that elicited depressor and bradycardic responses did not elicit a response when injected intravenously, indicating that leakage, if any, of E-2 from the microinjection site into the peripheral circulation was not responsible for the responses.
A bell-shaped curve was observed for the depressor and bradycardic responses when only one concentration of E-2 was microinjected into the mNTS in each animal. Other authors reported similar concentration-response curves using other chemical agents. For example, Criscione et al. (4), using microinjections of carbachol and acetylcholine, and Ciriello and Zhang (2), using neurotensin microinjections into the mNTS, reported similar concentration-response curves. Variations, if any, in the placement of microinjections were not responsible for the bell-shaped concentration-response curves, because when different concentrations of E-2 were microinjected at 20-min intervals at the same site in the same animal without withdrawal of the micropipette, the responses were.

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similar to those observed in experiments in which different concentrations were injected individually as single injections in different rats.

The depressor and bradycardic responses to microinjections of E-2 into the mNTS were mediated via \( \mu_1 \)-opioid receptors, because naloxonazine, a specific antagonist for \( \mu_1 \)-opioid receptors (26), abolished the depressor and bradycardic responses. On the other hand, microinjections of aCSF did not alter the responses to subsequent microinjections of E-2. Microinjections of naloxonazine by itself did not elicit any re-

Fig. 9. Spike-train analysis of neuron in Fig. 8. A: basal firing of neuron. B: effect of phenylephrine injected intravenously 3 min after recording in A; blood pressure and neuronal firing were increased, and this effect lasted for 5 s. C: application of L-Glu (5 mmol/l, 5 nl) to neuron increased its firing, which peaked at 0.1 s and lasted for 0.5 s. D: application of aCSF 5–6 s after Glu did not alter basal firing rate and did not affect responses to subsequent application of E-2. E: application of E-2 (0.2 mmol/l, 5 nl) increased neuronal firing, which reached a peak within 0.1 s and lasted for 0.4 s. F: application of D-AP7, followed 2–3 s later by NBQX, decreased firing of the neuron within 10–15 s; response lasted for 40–50 s. G: subsequent application of E-2 failed to elicit a response. H: response to application of E-2 recovered within 3 min. **Time bar for A and C–H.

Fig. 10. Excitatory effect of carbachol on mNTS neuron. A and G: basal firing rate (20 spikes/s) of 1 mNTS neuron. B and H: bolus injection of phenylephrine (3 \( \mu \)g/kg iv) increased MAP (40 mmHg) and neuronal firing (34 spikes/s). C and I: application of aCSF (5 nl) did not alter basal rate of neuronal firing and did not alter responses to subsequent application of carbachol. D and J: direct application of carbachol (0.5 mmol/l, 5 nl) increased neuronal firing (40 spikes/s). E and K: direct application of D-AP7 (5 mmol/l, 5 nl), followed 2–3 s later by NBQX (2 mmol/l, 5 nl), decreased firing rate of the neuron. F and L: application of carbachol 2–3 s after application of D-AP7 and NBQX elicited increase in neuronal firing. Firing rates reflect peak increases. In A and C–F, arrows indicate ejection of different agents on mNTS neurons; in B, arrow indicates intravenous injection of PE. EAA, excitatory amino acid.
response, suggesting that μ₁-opioid receptors in the mNTS are not endogenously active in the rat. Our observation with naloxonazine is in agreement with prior reports in which microinjections of naloxone into the NTS elicited no cardiovascular effects (9). Endogenous μ₁-opioid receptors may become activated in yet unidentified situations and exert modulatory influences on cardiovascular regulation. In the concentrations used, naloxonazine did not exert nonspecific effects, because it did not alter responses to a nonopioid receptor agonist such as L-Glu.

Our observations that microinjections of E-2 into the mNTS elicit depressor and bradycardic responses are in agreement with reports in which microinjections of β-endorphin into the mNTS of rats elicited depressor responses (9, 24). On the other hand, in other reports, microinjections of opioids into the NTS elicited pressor responses (9, 14). The differences between our responses and those reported by others (9, 14) may also be due to involvement of different subtypes of μ-receptors. This contention is supported by the reports that antinociceptive effects of DAMGO, E-2, and endomorphin-1 are mediated via different μ-receptor subtypes (28). Possible placement of our microinjections in different regions of the NTS did not account for the differences between our responses and those of others (9, 14).

The bradycardia elicited by microinjections of E-2 into the mNTS was mediated by the activation of the parasympathetic innervation to the heart. This conclusion was based on our observations that ipsilateral vagotomy significantly attenuated, while bilateral vagotomy completely abolished, the bradycardic responses to microinjections of E-2 into the mNTS. The
mechanism of bradycardia can be explained as follows. Disinhibition caused by microinjections of E-2 into the mNTS results in the activation of secondary mNTS neurons via ionotropic glutamate receptors. The presence of an excitatory projection from the mNTS to the nucleus ambiguus has been reported (21). Activation of nucleus ambiguus neurons may increase the parasympathetic activity to the heart via the vagus nerves and elicit bradycardia. Participation of the dorsal nucleus of the vagus in mediating bradycardia cannot be excluded, because this nucleus is located just ventral to the mNTS and may be activated by microinjections of E-2 into the mNTS. Although the depressor responses to microinjections of E-2 into the mNTS were decreased by ipsilateral as well as bilateral vagotomy, the attenuation in these responses was not statistically significant.

The depressor and bradycardic responses elicited by microinjections of E-2 were abolished after the blockade of ionotropic glutamate receptors in the mNTS. In these experiments, tachyphylaxis was not responsible for the lack of responses of E-2, because the interval between the two microinjections of E-2 was ≥20 min; in other experiments, repeated microinjections of E-2 into the mNTS did not elicit tachyphylaxis when the interval between injections was 20 min.

The specificity of the ionotropic glutamate receptor antagonists used in this study (d-AP7 and NBQX) was indicated by the observation that these agents did not alter the responses to microinjections of another unrelated agonist, carbachol (4), into the mNTS.

Unilateral blockade of ionotropic glutamate receptors in the mNTS with a combination of d-AP7 and NBQX did not elicit an increase in baseline BP. This observation is unexpected, considering that glutamate is believed to be the neurotransmitter at the terminals of peripheral afferents in the mNTS (29, 35). However, our observation is in agreement with a report in which blockade of ionotropic glutamate receptors by unilateral microinjections of kynurenic acid into the mNTS did not elicit a significant increase in baseline BP (8). Perhaps compensatory mechanisms in the contralateral mNTS prevent the baseline BP from rising. Consistent with this explanation are the reports that bilateral blockade of glutamate receptors in the mNTS increases the baseline BP (10, 17, 22). Furthermore, blockade of ionotropic as well as metabotropic glutamate receptors in the mNTS may be necessary to elevate baseline BP (8).

Microinjections of d-AP7 and NBQX did not attenuate cardiovascular responses to microinjections of l-Glu. This observation is in agreement with other earlier reports. For example, Leone and Gordon (17), Pawloski-Dahm and Gordon (22), and Talman (see Table 1 in Ref. 34) reported that concentrations of kynurenic acid (which blocked NMDA and kainate glutamate receptors) did not attenuate the responses to exogenously microinjected l-Glu into the mNTS, whereas the effects of endogenously released glutamate (e.g., by aortic nerve stimulation) were abolished. An explanation of these observations can be hypothesized as follows. It has been reported that ionotropic glutamate receptors may be located within the glutamatergic synapse, whereas the metabotropic glutamate receptors may be located in the perisynaptic region (32) (Fig. 12). Thus ionotropic glutamate receptors are readily accessible to glutamate released endogenously from nerve terminals, and blockade of ionotropic glutamate receptors is more likely to abolish the effects of endogenously released glutamate. On the other hand, exogenously microinjected glutamate may reach ionotropic receptors within the synapse as well as metabotropic receptors in the perisynaptic region. Therefore, blockade of ionotropic glutamate receptors alone is not sufficient to abolish the effects of endogenous glutamate; blockade of ionotropic and metabotropic receptors may be necessary to abolish the response to exogenously injected glutamate into the NTS (8).

The blockade of GABA A and GABA B receptors in the mNTS by gabazine and 2-hydroxsaclofen, respectively, also abolished the depressor and bradycardic responses to microinjections of E-2 into the mNTS. Because the interval between microinjections of E-2 was 20 min, the lack of response to E-2 after GABA receptor blockade was not due to tachyphylaxis. The specificity of GABA receptor blockers was indicated by the observations that these agents did not alter the depressor responses elicited by microinjections of l-Glu into the mNTS.

![Fig. 12. Hypothetical model of a glutamatergic synapse.](http://ajpregu.physiology.org/doi/10.1152/ajpregu.00335.2004)

Ionotropic glutamate receptors [NMDA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate receptors] are located on postsynaptic neuron within synapse and are accessible to endogenously released glutamate (Glu). Metabotropic glutamate receptors (mGlurRs) are located in perisynaptic region. These receptors are accessible to exogenously microinjected glutamate but not endogenously released Glu. GABAergic neurons located adjacent to glutamatergic synapse. GABA released from these neurons acts on postsynaptic glutamate terminals and inhibits release of glutamate. Exogenously microinjected E-2 acts on µ-opioid receptors (µ-R) located on the GABAergic neurons and inhibits them. GABAergic inhibition of glutamate release from the terminals is removed (disinhibition). There is an increase in glutamate release from glutamatergic terminals. Endogenously released glutamate excites the postsynaptic neuron via ionotropic glutamate receptors and elicits depressor and bradycardic responses.
Neuronal recording experiments confirmed our results using the microinjection technique. The involvement of the neuron under investigation in cardiovascular function was confirmed by excitation of the neuron when systemic BP was temporarily increased to stimulate baroreceptors by an intravenous bolus injection of phenylephrine. That the activity was recorded from a neuron, rather than a fiber of passage, was confirmed by excitation of the neuron by direct application of L-Glu, which stimulates neuronal cell bodies but not fibers of passage. Excitation of neurons by E-2 was not due to any nonspecific effects of pressure applications, because application of aCSF did not elicit any changes in the neuronal firing.

The excitatory effect of E-2 on the mNTS neurons was prevented by prior application of naloxonazine, indicating that the responses were indeed mediated via \( \mu \)-opioid receptors. Direct application of aCSF to mNTS neurons did not alter the responses to subsequent applications of E-2. Naloxonazine by itself did not elicit any response, suggesting that \( \mu \)-opioid receptors on the mNTS neurons involved in cardiovascular regulation are not endogenously active in the rat.

E-2-induced excitation of the neurons was completely abolished by prior applications of ionotropic glutamate receptor antagonists (D-AP7 and NBQX), indicating that ionotropic glutamate receptors mediated the actions of E-2. The firing rate of the neurons in response to direct application of another unrelated agonist, carbacchol, was not altered by the application of D-AP7 and NBQX, indicating that these ionotropic glutamate receptor antagonists did not exert any nonspecific effects. Direct application of D-AP7 and NBQX to single mNTS neurons elicited a significant reduction in the firing of these neurons within 10–15 s. Reduction of neuronal firing after the application of D-AP7 and NBQX was expected, considering that glutamate released from the baroreceptor terminals in the mNTS, in response to an increase in systemic BP, excites the mNTS neurons. However, inhibition of one neuron is not sufficient to elicit an increase in baseline BP. A number of neurons have to be inhibited simultaneously and compensatory effects of contralateral mNTS have to be eliminated by making lesions or inhibiting neurons in the other NTS to elicit an increase in baseline BP.

As noted earlier, E-2-induced responses to microinjections into the mNTS or direct applications to mNTS neurons were abolished by the blockade of ionotropic glutamate or GABA receptors. Taken together, these observations prompt our hypothesis that E-2 may enhance the release of glutamate into the mNTS via the mechanism of “disinhibition.” Immunohistochemical studies have demonstrated the presence of GABAergic neurons (16, 19) and terminals (36) in the NTS. Glutamatergic nerve terminals of peripheral baroreceptor and cardiopulmonary afferents are known to make their first synapse in the mNTS (1, 29, 35). The intrinsic GABAergic neurons in the mNTS may inhibit the release of glutamate from their terminals (Fig. 12). Consistent with this notion is the observation that baclofen (a GABA\(_B\) receptor agonist) may act presynaptically and inhibit the neuronal release of glutamate (7). There is a general consensus that opioid peptides interact with G protein-coupled opioid receptors and usually exert inhibitory effects on neurons (6, 15, 38). Thus inhibition of GABAergic neurons by E-2 via \( \mu \)-receptors may result in an increase in the neuronal release of glutamate. This may be one reason why E-2-induced depressor and bradycardic responses were no longer observed when the GABA receptors in the mNTS were previously blocked by 2-hydroxyasaclofen and gabazine. Furthermore, blockade of ionotropic receptors by D-AP7 and NBQX also resulted in the abolition of E-2-induced depressor and bradycardic responses.

The mechanism of E-2-induced bradycardia can also be explained by the disinhibition caused by microinjections of E-2 into the mNTS, which may result in the activation of secondary NTS neurons via ionotropic glutamate receptors. The presence of an excitatory projection from the NTS to the nucleus ambiguus has been reported (21). Activation of nucleus ambiguus neurons activates the parasympathetic activity to the heart via the vagus nerves, and bradycardia is elicited. Therefore, bilateral vagotomy abolished the E-2-induced bradycardia.

In summary, the results of this investigation, using microinjection and single-unit recording techniques, show for the first time that microinjections of E-2 into the mNTS elicit depressor and bradycardic responses via ionotropic glutamate receptors. This conclusion was based on the observation that the depressor and bradycardic effects of microinjections of E-2 into the mNTS were completely blocked when the ionotropic glutamate receptors were specifically blocked. The responses to E-2 were also abolished by GABA receptor antagonists. It was concluded that E-2 may inhibit GABAergic neurons in the mNTS. GABAergic neurons may normally inhibit the release of glutamate from the terminals of peripheral afferents in the mNTS. Inhibition of GABAergic neurons may, therefore, result in an increase in the release of glutamate in the mNTS, which, in turn, may elicit depressor and bradycardic responses via activation of NMDA and non-NMDA receptors on the secondary mNTS neurons.

Perspectives

It is well established that in the rat the mNTS is the site where peripheral baroreceptor and cardiopulmonary afferents make their primary synapse (1, 29, 35). The presence of opioid receptors in the mNTS is also well documented (9). The results of the present study indicating that microinjections of E-2 into the mNTS elicit depressor and bradycardic responses that are mediated via ionotropic glutamate receptors are novel. The physiological significance of these results may lie in the report showing that microinjections of glutamate into the mNTS elicit antinociception in addition to cardiovascular effects (27). On the basis of these reports, it may be hypothesized that E-2 is released in the mNTS in response to visceral nociceptive stimuli. The released E-2 may inhibit GABAergic neurons in the mNTS that normally inhibit the release of glutamate from their terminals. Thus glutamate release may be increased in the mNTS via the disinhibition caused by E-2. The increase in glutamate release in the mNTS may then activate neural pathways that may suppress nociceptive responses elicited by the visceral nociceptive stimuli (27). This hypothesis remains to be tested.

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