Attenuation of aortic baroreflex responses by microinjections of endomorphin-2 into the rostral ventrolateral medullary pressor area of the rat

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Kasamatsu, Ken, and Hreday N. Sapru. Attenuation of aortic baroreflex responses by microinjections of endomorphin-2 into the rostral ventrolateral medullary pressor area of the rat. Am J Physiol Regul Integr Comp Physiol 289: R59–R67, 2005. First published February 17, 2005; doi:10.1152/ajpregu.00007.2005.—The presence of μ-opioid receptors and endorphins has been demonstrated in the general area encompassing the rostral ventrolateral medullary pressor area (RVLM). This investigation was carried out to test the hypothesis that endorphins in the RVLM may have a modulatory role in regulating cardiovascular function. Blood pressure and heart rate (HR) were recorded in urethane-anesthetized male Wistar rats. Unilateral microinjections of endomorphin-2 (0.0125–0.5 mmol/l) into the RVLM elicited decreases in mean arterial pressure (16–30 mmHg) and HR (12–36 beats/min), which lasted for 2–4 min. Bradycardia was not vagally mediated. The effects of endomorphin-2 were mediated via μ-opioid receptors because prior microinjections of naloxonazine (1 mmol/l) abolished these responses; the blocking effect of naloxonazine lasted for 15–20 min. Unilateral stimulations of aortic nerve for 30 s (at frequencies of 5, 10, and 25 pulses/s; each pulse 0.5 V and 1-ms duration) elicited depressor and bradycardic responses. These responses were significantly attenuated by microinjections of endomorphin-2 (0.2 and 0.4 mmol/l). The inhibitory effect of endomorphin-2 on baroreflex responses was prevented by prior microinjections of naloxonazine. Microinjections of naloxonazine alone did not affect either baseline blood pressure and HR or baroreflex responses. These results indicate that endomorphin-2 elicits depressor and bradycardic responses and inhibits baroreflex function when injected into the RVLM. These effects are consistent with the known hyperpolarizing effect of opioid peptides on RVLM neurons.

RVL (19, 27). From these reports, it was hypothesized that endorphins may play a role in the mediation and/or modulation of cardiovascular function in the RVLM. Except for one preliminary study from this laboratory (33), there is no report in the literature in which the role of endorphins in the cardiovascular regulation at the level of RVLM has been studied in vivo. In this paper, we describe the mechanism of cardiovascular effects of E-2 in the RVLM and the effect of μ-opioid-receptor activation on the aortic baroreflex.

MATERIALS AND METHODS

General procedures. Experiments were done in adult male Wistar rats (Charles River Laboratories, Wilmington, MA) weighing 300–350 g (n = 56). 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 experimental procedures were performed in accordance with the guidelines for animal experimentation described in the APS “Guiding Principles for Research Involving Animals and Human Beings,” and the Institutional Animal Care and Use Committee of this university approved the experimental protocols. The number of animals used was the minimum required for statistical analyses of the data, and every effort was made to minimize any suffering to the animals.

Details of general procedures used in this study have been described in our previous publication (16). Briefly, the trachea, one of the femoral veins, and arteries were cannulated under isoflurane anesthesia (3% in 100% oxygen) administered via a nose mask. The animals were artificially ventilated, and blood pressure (BP), heart rate (HR), and rectal temperature were monitored by standard techniques. Urethane (1.2–1.4 g/kg) was injected intravenously in six or seven aliquots at 2-min intervals, and tracheal inflation of isoflurane was discontinued. The depth of anesthesia was established by pinching the hind paw 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. All of the tracings were recorded on a polygraph (Grass Instruments, model 7D).

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

Microinjection technique. A ventral approach was used to identify the RVLM (39). The rats were placed in a supine position in a stereotaxic instrument (model 1430, David Kopf Instruments, Tujunga, CA), and the head was fixed using the ear and bite bars. The larynx, esophagus, and underlying muscles were removed to expose the basal aspect of the occipital bone, and a window (6 mm wide and 6 mm long) was created in this bone to expose the ventral aspect of the medulla. The bite bar was adjusted so that the ventral surface of the immediatly subjacent bone was visible.

THE IMPORTANCE OF ROSTRAL VENTROLATERAL MEDULLARY PRESSOR AREA (RVLM) IN THE REGULATION OF CARDIOVASCULAR FUNCTION IS WELL ESTABLISHED (12, 18, 29, 35). ELECTROPHYSIOLOGICAL AND ANATOMICAL EVIDENCE SHOWS THAT SYMPATHOEXCITATORY NEURONS LOCATED IN THE RVLM SEND DIRECT MONOSYNAPTIC PROJECTIONS TO THE INTERMEDIO-LATERAL CELL COLUMN OF THE SPINAL CORD (6, 12). IT IS ALSO WELL KNOWN THAT RVLM IS IMPORTANT IN MEDiating the baroreceptor, chemoreceptor, and cardiopulmonary reflex responses (12, 18, 29, 35).

The presence of μ-opioid receptors has been reported in the general area encompassing the RVLM (2, 9, 20). Two tetrapeptides [endorphin-1 and endorphin-2 (E-2)], isolated from human cortex and bovine hypothalamus, 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 (14, 40). The presence of endorphin-like immunoreactivity has been demonstrated in the general region of the RVLM (19, 27). From these reports, it was hypothesized that endorphins may play a role in the mediation and/or modulation of cardiovascular function in the RVLM. Except for one preliminary study from this laboratory (33), there is no report in the literature in which the role of endorphins in the cardiovascular regulation at the level of RVLM has been studied in vivo. In this paper, we describe the mechanism of cardiovascular effects of E-2 in the RVLM and the effect of μ-opioid-receptor activation on the aortic baroreflex.

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medulla was in a horizontal plane. Two to four barreled glass micropipettes (tip size of 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 on a picospritzer (General Valve, Fairfield, NJ). One of the barrels contained L-glutamate (L-Glu), and the contents of the other barrels varied according to the requirements of the experiment being conducted. For example, in concentration-response experiments, one barrel contained L-Glu (5 mmol/l), the second one contained India ink, and the remaining two barrels contained different concentrations of E-2 selected at random. Similarly, in experiments for testing possible tachyphylaxis of response, three barreled micropipettes were used: one barrel contained L-Glu, the second contained E-2, and the third one contained India ink. The coordinates for the RVLM were as follows: 1.3–1.7 mm rostral to the confluence of vertebral arteries, 1.7–1.9 mm lateral to the midline, and 0.9–1.1 mm deep from the ventral medullary surface. The rostrocaudal reference point used in this ventral lateral to the midline, and 0.9–1.1 mm deep from the ventral medullary surface. The rostrocaudal reference point used in this ventral lateral to the midline, and 0.9–1.1 mm deep from the ventral medullary surface. The rostrocaudal reference point used in this ventral lateral to the midline, and 0.9–1.1 mm deep from the ventral medullary surface.

In previous publications (23), in this study, typical sites of microinjections were marked by a unilateral microinjection (100 nl) of diluted India ink. Standard techniques were used for perfusion of the animals and tissue fixation (16). Serial sections of the medulla were cut (30 μm) in a cryostat, mounted on slides, and stained with cresyl violet; the microinjection 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 (26).

Results

Concentration response of E-2. As mentioned in MATERIALS AND METHODS, the RVLM was always identified by microinjections of L-Glu (5 mmol/l), which stimulates neurons but not fibers of passage. To select appropriate volumes of microinjections, E-2 (0.2 mmol/l) was microinjected into the same site of the RVLM in 100- and 50-nl volumes (n = 5). The E-2-induced decreases in MAP were 34 ± 4.8 and 21 ± 3.7 mmHg for 100- and 50-nl volumes, respectively (P < 0.05). Similarly, the E-2-induced decreases in HR were 28 ± 8.0 and 12 ± 4.9 beats/min for 100- and 50-nl volumes, respectively (P < 0.05). Because the responses to E-2 were significantly greater when the volume of microinjection was 100 nl, this volume was selected for all other microinjections, including those of L-Glu.

Concentration response for E-2 was studied in the RVLM (1.3–1.7 mm rostral to the confluence of vertebral arteries, 1.7–1.9 mm lateral to the midline, and 0.9–1.1 mm deep from the ventral medullary surface) where microinjections of L-Glu (5 mmol/l, 100 nl) induced pressor (23 ± 1.7 mmHg) and tachycardic (20 ± 3.4 beats/min) responses. The interval between the microinjections of L-Glu and E-2 was at least 5 min. Microinjections of E-2 into the RVLM (n = 5) elicited decreases in MAP (Fig. 1A) and HR (Fig. 1B). Maximal depressor and bradycardic responses were elicited by a 0.2 mmol/l concentration. The onset and durations of the responses to microinjections of E-2 (0.2 mmol/l) were 5 s and 2–4 min, respectively, and the peak effect was observed at 1–2 min. In these experiments, three concentrations of E-2, selected at random, were microinjected into the RVLM of each animal. The interval between different microinjections of E-2 was 20 min to avoid tachyphylaxis. A concentration of E-2 that elicited maximum responses (0.2 mmol/l) when microinjected into the RVLM did not elicit a response when injected intravenously. Absence of tachyphylaxis to repeated microinjections of E-2 was tested in another group of rats (n = 4). The concentration of E-2 that elicited maximal cardiovascular responses (0.2 mmol/l) was microinjected into the RVLM 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 24 ± 5.5, 28 ± 5.9, and 26 ± 1.3 mmHg, respectively (P > 0.05). The decreases in HR in response to these microinjections of E-2 were 18 ± 2.5, 18 ± 2.5, and 15 ± 2.8 beats/min, respectively (P > 0.05). Thus no tachyphylaxis of responses was observed with repeated microinjections of E-2.

Role of vagus in E-2-induced bradycardia. E-2-induced decreases in MAP and HR before and after bilateral vagotomy are shown in Fig. 1, C and D, respectively; bilateral vagotomy did not significantly (P > 0.05) alter the depressor and bradycardic responses to E-2 in the RVLM.

μ-Opioid receptors mediate E-2-induced responses. A tracing showing the effects of μ-receptor blockade on E-2 induced responses in the RVLM is presented in Fig. 2. The RVLM region from which pressor and tachycardic responses were elicited was identified by a microinjection of L-Glu (5 mmol/l, Fig. 2A). Within 5 min, microinjections of aCSF (100 nl) at the same site elicited no responses (Fig. 2B). After an interval of 2

Fig. 1. A: concentration response for mean arterial pressure (MAP). Microinjections of endomorphin-2 (E-2; 0.0125, 0.025, 0.05, 0.2, and 0.5 mmol/l) into the rostral ventrolateral medullary pressor area (RVLM) (n = 5 for each concentration) elicited decreases in MAP (16 ± 2.5, 19 ± 2.5, 20 ± 1.6, 30 ± 1.6, and 26 ± 2.5 mmHg, respectively). B: concentration response for heart rate (HR). Microinjections of E-2 (0.0125, 0.025, 0.05, 0.2, and 0.5 mmol/l) into the RVLM elicited decreases in HR (12 ± 2.0, 18 ± 3.0, 18 ± 3.7, 36 ± 2.5, and 28 ± 3.7 beats/min, respectively). *Depressor and bradycardic responses at the concentration of 0.2 mmol/l were significantly greater (P < 0.05) than the responses at the concentrations of 0.0125, 0.025, and 0.05 mmol/l. C: decreases in MAP elicited by E-2 before (26 ± 2.5 mmHg) and after (26 ± 3.7 mmHg) bilateral vagotomy (Vag-X) were not statistically different (P > 0.05). D: decreases in HR elicited by E-2 before (28 ± 3.7 beats/min) and after (24 ± 2.5 beats/min) bilateral vagotomy were also not statistically different (P > 0.05). bpm, Beats/min.

Fig. 2. Blockade of E-2 responses by naloxonazine. Top trace: pulsatile arterial pressure (PAP). Middle trace: MAP. Bottom trace: HR. A: identification of RVLM; microinjection of L-glutamate (L-Glu; 5 mmol/l) into the RVLM elicited increases in MAP and HR. B: microinjection of artificial cerebrospinal fluid (aCSF) 5 min after L-Glu did not elicit a response and did not alter the 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: naloxonazine (1 mmol/l) microinjected 20 min after the recovery of E-2-induced responses did not elicit a response by itself, and microinjection of E-2, 2 min after naloxonazine, failed to elicit a response. E: recovery of cardiovascular responses to E-2 after an interval of 20 min.
min, microinjection of E-2 (0.2 mmol/l) at the same site elicited decreases in MAP and HR (Fig. 2C), which lasted for 2 min. Twenty minutes after the recovery of responses, naloxonazine (1 mmol/l) was microinjected at the same site. Microinjection of naloxonazine alone elicited no cardiovascular responses (Fig. 2D). Two minutes after the microinjection of naloxonazine, microinjection of E-2 (0.2 mmol/l) failed to elicit any response (Fig. 2D). The blocking effect of naloxonazine lasted for 15–20 min (Fig. 2E). The lack of responses to E-2 was not due to tachyphylaxis because repeated microinjections of E-2 at 20-min intervals did not exhibit tachyphylaxis as described earlier. This concentration of naloxonazine (1 mmol/l) was selected for blockade of E-2 responses because a smaller concentration of naloxonazine (0.5 mmol/l) did not significantly block the responses to E-2 (0.2 mmol/l) (n = 5). Group data for the effects of naloxonazine (1 mmol/l) on E-2-induced effects on MAP and HR responses are presented in Fig. 3, A and B, respectively; naloxonazine completely abolished depressor and bradycardic responses elicited by E-2. Naloxonazine did not affect the pressor (Fig. 3C) and tachycardic (Fig. 3D) responses elicited by microinjections of L-Glu (5 mmol/l) into the RVLM; the lack of effects of naloxonazine (1 mmol/l) on the responses elicited by L-Glu indicated that this antagonist did not exert any nonspecific effects in the RVLM.

E-2 attenuates the responses to aortic nerve stimulation. In one group of rats (n = 5), responses to repeated aortic nerve stimulations were monitored. The decreases in MAP during the first, second, and third test were 19 ± 3.3, 17 ± 2.6, and 17 ± 2.0 mmHg, respectively, at 5 pps; 29 ± 4.4, 27 ± 2.0, and 27 ± 3.0 mmHg, respectively, at 10 pps; and 36 ± 4.8, 34 ± 2.9, and 38 ± 2.6 mmHg, respectively, at 25 pps. Similarly, the decreases in HR during the first, second, and third test were 40 ± 8.4, 34 ± 10.3, and 34 ± 9.8 beats/min, respectively, at 5 pps; 52 ± 13.2, 43 ± 9.7, and 52 ± 13.2 beats/min, respectively, at 10 pps; and 60 ± 15.2, 53 ± 13.6, and 58 ± 16.2 beats/min, respectively, at 25 pps. Thus MAP and HR responses were not significantly different (P > 0.05) for at least three repeated cycles of electrical stimulations (each cycle consisting of three stimulus frequencies).

In another group of rats (n = 7), before E-2 was microinjected into the RVLM, the decreases in MAP in response to electrical stimuli of 5, 15, and 25 pps were 16 ± 1.8, 28 ± 1.8, and 32 ± 2.4 mmHg, respectively, and the decreases in HR in response to the same stimulations were 27 ± 5.2, 37 ± 8.8, and 46 ± 4.8 beats/min, respectively. The onset of responses for each stimulus was immediate, whereas the peak effects were elicited within 4–5 s after the simulation. The MAP and HR recovered to basal levels within 4–5 s after the cessation of the stimulation. In the same group of rats, 10 min after the last stimulus was applied, RVLM was identified with L-Glu microinjection and E-2 (0.2 mmol/l; maximally effective concentration) was microinjected at the same site. After 2–4 min, when the depressor and bradycardic responses to E-2 abated and BP and HR recovered to basal levels, the aortic nerve stimulations were again applied. After the microinjection of E-2 into the RVLM, the decreases in MAP in response to electrical stimuli of 5, 15, and 25 pps were 11 ± 1.7, 16 ± 2.6, and 22 ± 2.4 mmHg, respectively, and the decreases in HR in response to the same stimulations were 15 ± 3.3, 20 ± 3.1, and 24 ± 3.7 beats/min, respectively. Thus E-2 significantly (P < 0.05)
attenuated cardiovascular responses to the aortic nerve stimulation. After a period of 20 min, the aortic nerve was again stimulated. The decreases in MAP in response to electrical stimulations of 5, 15, and 25 pps were 16 ± 1.3, 24 ± 3.8, and 30 ± 2.8 mmHg, respectively, and the decreases in HR in response to the same stimulations were 27 ± 8.8, 37 ± 8.8, and 40 ± 5.8 beats/min, respectively. Thus, the responses to aortic nerve stimulation recovered within 20 min from the attenuation caused by E-2 microinjection.

In a different group of rats (n = 8), the effects of microinjections of a higher concentration (0.4 mmol/l) of E-2 into the RVLM on the responses to electrical stimulations of the aortic nerve were studied. Figure 4 shows the decreases in MAP and HR in response to electrical stimuli at 5, 10, and 25 pps before, 2 min after, and 20 min after the microinjection of E-2 into the RVLM. Microinjections of E-2 significantly (P < 0.01) attenuated the baroreflex responses. Recovery of baroreflex responses was observed after 20 min. Typical tracings showing BP and HR responses to aortic nerve stimulations at different frequencies before the microinjection of E-2 into the RVLM, attenuation of these responses after microinjection of E-2 (0.4 mmol/l), and recovery of these responses after an interval of 20 min are presented in Fig. 5, A–C, respectively.

In another group of rats (n = 5), the effect of microinjection of a µ-receptor antagonist (naloxonazine) into the RVLM on the aortic baroreflex was studied. Before microinjection of naloxonazine into the RVLM, the decreases in MAP and HR (Fig. 6) in response to electrical stimulations of 5, 15, and 25 pps were noted. Ten minutes after the last stimulus was applied, RVLM was identified with l-Glu microinjection, and naloxonazine (1 mmol/l) was microinjected at the same site; this antagonist elicited no cardiovascular responses. Two minutes after the microinjection of naloxonazine, the aortic nerve stimulation was repeated, and decreases in MAP and HR (Fig. 6) in response to same electrical stimulations of the aortic nerve were observed; blockade of µ-receptors in the RVLM did not alter baroreflex responses. In the same group of rats, naloxonazine (1 mmol/l) was microinjected into the RVLM before microinjections of E-2 (0.4 mmol/l) into the same site, and the responses to aortic nerve stimulation were studied. E-2 did not elicit any cardiovascular response after prior microinjections of naloxonazine. Moreover, E-2 did not elicit attenuation of aortic baroreflex responses when µ-receptors were previously blocked by naloxonazine (Fig. 6).

Histology. The RVLM sites, where microinjections of E-2 elicited inhibitory effects, were marked in six rats. A typical RVLM site marked with India ink (100 nl) is shown in Fig. 7A (arrow). Figure 7B represents a composite diagram of these sites. The sites marked with India ink were located in the RVLM, 1.3–1.7 mm rostral to the confluence of vertebral arteries on the ventral surface, 1.7–1.9 mm lateral to the midline, and 0.9–1.1 mm deep from the ventral medullary surface.

**DISCUSSION**

The main findings of this study are that microinjections of E-2 into the RVLM of the rat elicited depressor and bradycardic responses and attenuated reflex responses (i.e., fall in BP and HR) to aortic nerve stimulation. As mentioned in RESULTS, a 100-nl volume was selected for microinjections because responses to l-Glu or E-2 using this volume were greater than those elicited by 50-nl volume microinjections. The radius of the diffusion area with a 100-nl volume microinjection has been estimated to be 0.288 mm (24). It may be argued that observed responses were elicited by diffusion of the injectate to adjacent areas. However, it appears that the responses elicited by L-Glu or E-2 were specifically elicited from the RVLM because when either L-Glu or E-2 was microinjected more medially (e.g., 1.4–1.5 mm lateral to the midline), no responses were elicited. Moreover, microinjections of E-2 in an area caudal to the RVLM (e.g., 1.0 rostral to the confluence of
the vertebral arteries, 1.7–1.9 lateral to the midline, and 0.9–1.1 mm deep from the ventral medullary surface; an area within caudal ventrolateral medullary depressor area, generally known as CVLM) elicited pressor and tachycardic responses instead of depressor and bradycardic responses (unpublished observations). Local distortion of brain tissue or any nonspecific effects were not responsible for these observations because microinjections of aCSF into the RVLM did not elicit any response. Concentrations of microinjections of E-2 into the RVLM 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 observed responses. The effects of E-2 were mediated via µ-opioid receptors because prior microinjections of naloxonazine prevented E-2-induced effects. The specificity of naloxonazine for µ-opioid receptors has been demonstrated in our previous report (16).

Our observations that microinjections of E-2 into the RVLM elicit depressor responses are in agreement with earlier reports in which other opioid-receptor agonists (e.g., enkephalins) were microinjected into the RVLM (32). The bradycardia elicited by microinjections of E-2 into the RVLM was not mediated via the activation of the parasympathetic innervation to the heart because bilateral vagotomy did not abolish these responses. The depressor and bradycardic responses elicited by E-2 into the RVLM may represent inhibitory effects of this opioid-receptor agonist on sympathoexcitatory neurons in this region. The autonomic regions in the thoracic and upper lumbar spinal are known to receive excitatory input from the sympathoexcitatory RVLM neurons (6, 12, 29, 37). E-2 microinjected into the RVLM may hyperpolarize sympathoexcitatory neurons by activating µ-receptors located on them; the presence of µ-opioid receptors on bulbar spinal RVLM neurons has been demonstrated by immunohistochemical studies (2). The inhibitory effect of opioids on RVLM neurons is well documented (3, 8, 13). For example, Hayar and Guyenet (15) made whole cell recordings from RVLM neurons retrogradely labeled by prior injections of fluorescein isothiocyanate microspheres into the upper thoracic cord. These authors reported that endomorphin-1 and methionine enkephalin inhibited RVLM neurons. E-2-induced inhibition of RVLM neurons has been reported to be mediated via µ-opioid receptors (8). Inhibition of RVLM neurons by microinjections of E-2 is expected to decrease the excitatory input to the sympathetic preganglionic neurons located in the intermediolateral cell column of the spinal cord, causing bradycardia and hypotension via a decrease in the activity of sympathetic nerves innervating the heart and blood vessels, respectively. The proposed mechanism is consistent with our laboratory’s previous report (32) in which it was demonstrated that microinjections of opioids into the RVLM decrease greater splanchic nerve activity in the rat.

It is interesting to note the differences in responses to microinjections of E-2 into different regions of the medulla. For example, microinjections of E-2 into the medial subnucleus of the solitary tract (mNTS), at the level where baroreceptor afferents terminate, have been reported to elicit depressor and bradycardic responses instead of pressor and tachycardic responses expected on the basis of reported inhibitory effects of E-2 on neurons (16). These unexpected responses have been attributed to disinhibition caused by inhibitory effects of endomorphins on GABAergic neurons in the mNTS (16). Microinjections of E-2 into the RVLM did not involve this mechanism of action perhaps because very few GABAergic neuronal somata have been reported to be present in this region (20). In this context, it should be noted that, although GABAergic neurons are abundant in the ventral medulla, they are located in the rostral ventromedial medulla, a region located medial to the RVLM (36).

As mentioned earlier, microinjections of E-2 attenuated the depressor and bradycardic responses to aortic nerve stimulation. The aortic nerve in the rat contains predominantly baroreceptor afferents (25, 30, 31). Although the presence of a sparse

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**Fig. 5.** Tracing showing effect of E-2 microinjection on the aortic baroreflex. Arrangement of tracings same as in Fig. 2. A: decreases in blood pressure and HR elicited by aortic nerve stimulation at 5, 10, and 25 pps, before the microinjection of E-2 (0.4 mmol/l) into the RVLM. B: responses to aortic nerve stimulation at each frequency were attenuated by microinjection of E-2 into the RVLM C: inhibitory effect of E-2 on baroreflex responses lasted for 20 min. Solid bars at the bottom indicate the duration of aortic nerve stimulation.
population of chemoreceptor afferents in this nerve has been reported (5, 7), subsequent studies have shown that these afferents may not be of functional importance in the reflex regulation of cardiovascular function (17). Attenuation of reflex responses to aortic nerve stimulation by microinjections of E-2 was also mediated via \(\mu\)-opioid receptors because prior microinjections of naloxonazine prevented this effect. Attenuation of baroreflex responses by microinjections of E-2 into the RVLM is also consistent with inhibitory effects of opioids on RVLM neurons (3, 8, 13, 15). Our observation regarding the inhibitory effect of E-2 on aortic baroreflex is in agreement with a similar report in which microinjections of another \(\mu\)-opioid-receptor agonist ([D-Ala\(^2\),N-Me-Phe\(^4\),Gly-ol\(^5\)]-enkephalin; DAMGO) attenuated aortic baroreflex (21). Attenuation of baroreflex has also been reported when other opioids were microinjected into different areas of the baroreflex arc. For example, Gordon (11) reported that microinjections of DAMGO into the NTS attenuated the responses to electrical stimulation of the aortic nerve in the rat. Attenuation of the aortic baroreflex responses by microinjections of morphine into the NTS of the rabbit has also been reported (38). In agreement with these reports (11, 38), microinjections of a \(\mu\)-opioid-receptor antagonist (naloxonazine) alone into the NTS had no effect on the baroreflex, but this antagonist abolished the inhibitory effects of opioids on this reflex.

It is well established that activation of baroreceptor afferents results in the stimulation of secondary NTS neurons, which in turn activate GABAergic CVLM neurons, and baroreflex responses are mediated via the release of GABA from the terminals of these CVLM neurons in the RVLM (29). Activation of opioid receptors has been shown to have an inhibitory effect on the GABA release in in vitro rat brain slices (4, 34). It may, therefore, be argued that activation of opioid receptors on the terminals of GABAergic CVLM neurons in the RVLM may result in a decrease in the release of GABA, causing attenuation of baroreflex responses. However, in one of the
reports (20), a majority of \( \mu \)-opioid receptor-immunoreactive axon terminals in the RVLM were not labeled for GABA, leading to the suggestion that in the RVLM GABAergic terminals may not be targets of \( \mu \)-opioid-receptor agonists. Thus a decrease in GABA release from the terminals of CVLM GABAergic neurons is unlikely to be the mechanism by which E-2 microinjections into the RVLM attenuate aortic baroreflex. \( \mu \)-Opioid-receptor agonists have been shown to cause predominantly reductions in miniature postsynaptic currents in the RVLM reticulospinal neurons (15). From these observations, it may be hypothesized that \( \mu \)-opioid-receptor agonists may target glutamatergic terminals in the RVLM. However, this mechanism is unlikely to account for attenuation of baroreceptor reflex by microinjections of E-2 into the RVLM because activation of baroreflex involves primarily the release of GABA (not glutamate) in the RVLM (29).

In summary, the results of this investigation show for the first time that microinjections of E-2 into the RVLM elicit depressor and bradycardic responses and also attenuate aortic baroreflex. These responses are mediated via \( \mu \)-opioid receptors located in the RVLM.

Perspectives

It is well established that in the rat the RVLM mediates responses to stimulation of peripheral baroreceptor afferents (12, 29, 35). The presence of opioid receptors in the RVLM is also well documented (32). The physiological significance of our results, which indicate that microinjections of E-2 into the RVLM elicit depressor and bradycardic responses and attenuate aortic baroreflex, is not clear at the present time. Because microinjections of naloxonazine alone into the RVLM did not exert any cardiovascular effects, it is unlikely that endorphins play a role in the regulation of cardiovascular function under normal circumstances. However, opioidergic mechanisms may come into play in yet unidentified situations that affect cardiovascular function. One such possibility is that opioidergic mechanisms may be activated during nociception. Although these mechanisms remain to be investigated, the following scenario can be speculated. Electrical as well as chemical stimulation of NTS has been reported to produce antinociception (e.g., inhibition of nociceptive tail-flick reflex), hypotension, and bradycardia (1). In addition to other pathways, the projections from the NTS to nucleus raphe magnus and lateral reticular nucleus have been implicated in mediating these antinociceptive effects. In view of our results, hypotension and bradycardia accompanying the antinociceptive effects could be speculated to be mediated via release of endorphin in the RVLM. The presence of endorphin-containing cells in the NTS is consistent with this hypothesis (27). In this connection, it should be noted that the presence of projections to the RVLM from NTS cells containing other opioid peptides (e.g., enkephalins) has been reported (22).

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REFERENCES


