A projection from the ventral tegmental area to the periaqueductal gray involved in cardiovascular regulation

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Kirouac, Gilbert J., and Quentin J. Pittman. A projection from the ventral tegmental area to the periaqueductal gray involved in cardiovascular regulation. Am J Physiol Regulatory Integrative Comp Physiol 278: R1643–R1650, 2000.—Experiments were done in α-chloralose-anesthetized rats to determine a pathway mediating the cardiovascular depressor responses elicited from stimulation of the ventral tegmental area (VTA). The magnitude of the depressor responses elicited by glutamate stimulation (0.1 M/30 nl) of the VTA was examined after neuronal block produced by microinjections of lidocaine into ascending fiber bundles leaving the VTA to innervate the forebrain and thalamus. Bilateral microinjections of 1 µl of 4% lidocaine in the medial forebrain bundle (n = 6) and in the periventricular fibers of the midbrain (n = 5) did not attenuate the depressor response from stimulation of the VTA. Experiments were done using the anterograde tracer biotinylated dextran amine to identify descending projections from the VTA to cardiovascular centers in the brain stem. Examination of the nucleus of the solitary tract, ventrolateral medulla, and A5 catecholaminergic cell group revealed few or no fibers or terminals. Occasional fibers and some terminals were observed in the nucleus of raphe magnus, parabrachial nucleus, and locus ceruleus. A very dense bilateral projection was found to the ventrolateral periaqueductal gray (PAGvl) and dorsal raphe nucleus adjacent to the PAGvl. Bilateral injections of 4% lidocaine (n = 4) or 10 mM cobalt chloride (n = 5) into the PAGvl region attenuated the depressor responses elicited by stimulation of the VTA by ~50%. These experiments indicate that the depressor responses elicited from activation of the VTA are mediated in part by a pathway to a cardiovascular depressor area located in the PAGvl.

medial forebrain bundle; biotinylated dextran; lidocaine; blood pressure; pathway

THE VENTRAL TEGMENTAL AREA (VTA) located in the medial portion of the ventral mesencephalon and is formed in part by the A10 dopaminergic cell group (12) may be involved in the regulation of the cardiovascular system. For example, the activity of neurons in the VTA appears to be regulated by inputs from arterial baroreceptors (19). Kirouac and Ciriello (20) also demonstrated that stimulation of VTA neurons with the excitatory amino acid L-glutamate produced cardiovascular depressor responses that were mediated by an inhibition of sympathetic vasoconstrictor fibers to the vasculature and cardioacceleratory fibers to the heart. Intravenous administrations of the D2 dopamine-receptor antagonist raclopride attenuated the depressor responses from stimulation of the VTA, indicating that the responses were partially mediated by central dopaminergic projections from the VTA to some unknown area of the brain (20).

The VTA has widespread projections to many regions of the forebrain including several areas implicated in the regulation of the cardiovascular system (23). The majority of efferent projections from the VTA ascend in the medial forebrain bundle and terminate in widespread regions of the forebrain including the nucleus accumbens, diagonal band of Broca, septal nuclei, bed nucleus of the stria terminals, amygdala, and insular and prefrontal cortices (4, 23, 28). These forebrain regions are interconnected with other brain regions involved in regulation of the cardiovascular system, and stimulation of these regions has been shown to elicit changes in blood pressure and heart rate (13, 31).

A second group of efferent fibers from the VTA ascends as part of the periventricular fiber system that innervates parts of the medial thalamus and the habenular complex (4, 23, 28). The midline thalamus has been implicated in the regulation of the autonomic nervous system and may play a role in regulating cardiovascular reflexes (24, 29). Although less well described in the literature, descending projections innervate the inferior olivary complex, mesencephalic trigeminal nucleus, periaqueductal gray matter (PAG), and the cerebellum (4, 23, 28).

The present investigation was done to identify neuronal connections mediating the cardiovascular depressor responses elicited from stimulation of the VTA. Reversible neuronal block (22) of efferent fiber bundles leaving the VTA was used to determine the involvement of the pathways mediating the depressor responses. Microinjections of the local anesthetic lidocaine in fiber bundles from the VTA were used to chemically block nerve impulse traffic, whereas microinjections of cobalt chloride in terminal fields were used to block synaptic transmission in potential pathways mediating the depressor response (22). Tract-tracing experiments were carried out with the new and sensitive anterograde tracer biotinylated dextran amine (BDA) (14, 33) to identify descending projections from...
the VTA to cardiovascular regulatory centers in the midbrain and brain stem.

METHODS

Physiological Experiments

Surgical preparation. Experiments were conducted on 25 male Sprague-Dawley rats (300–450 g) according to guidelines of the Canadian Council on Animal Care. Rats were anesthetized using intravenous administrations of α-chloralose (60 mg/kg, after induction of anesthesia with administration of 0.3 ml/100 g ip of equithesin (20); supplemented by additional doses of 30 mg/kg of α-chloralose every ~1–2 h). Polyethylene catheters were inserted into the femoral artery and vein for the recording of arterial pressure and the administration of drugs, respectively. The trachea was cannulated, the animal was paralyzed with pancuronium bromide (Pavulon, Organon Canada, Toronto, Ontario; 1 mg/kg iv initially and additional doses of 0.5 mg/kg iv when necessary) and artificially ventilated with oxygen (Harvard Apparatus small animal ventilator model 683; 2.5 ml at a rate of 70 breaths/min). Rectal temperature was monitored and maintained at 35–37°C with a heating pad. Experiments were done with rats placed in a stereotaxic frame (Narishige) with the nose bar adjusted according to the stereotaxic atlas of Paxinos and Watson (25).

Data acquisition and analysis. Arterial blood pressure was recorded using a Statham P23XL pressure transducer and Gould transducer amplifier (model 13–4615–50), and heart rate was measured with a Gould electrocardiogram/Biotach amplifier (model 13–4615–65) that was triggered by the pressure pulse. Mean arterial pressure (MAP) was defined as the diastolic pressure plus one-third of the pressure pulse. The electronic signals for arterial pressure and heart rate were digitized using a Cambridge Electronic 1401 Plus interface Design (CED; Cambridge, UK), and the data were captured and analyzed on a computer (CED Spike2 data capture and analysis software).

Glass micropipettes with tip diameters of 30–50 µm containing a 0.1 M glutamate (sodium salt, Sigma) dissolved in 0.1 M PBS were lowered in the ventral midbrain on the right side (5.0–5.5 mm caudal to bregma, 0.8–1.3 mm lateral to the midline, and 6.5–8.5 ventral to the dura), and 30 nl of the glutamate solution were microinjected by the application of pressurized nitrogen pulses controlled by a pneumatic pump (Medical Systems, Great Neck, NY). The injected volumes were measured by the direct observation of the fluid meniscus in the micropipettes with a dissecting microscope fitted with a micrometer. Regions of the VTA were stimulated with glutamate to locate sites that produced depressor responses of at least 15 mmHg. A minimal distance of 300 µm separated each injection site. We have previously established that injection of the vehicle in the VTA does not produce changes in arterial pressure or heart rate (20). In preliminary experiments, we observed that repeated stimulation with glutamate of the same site within the VTA every 5 min leads to an attenuation in the magnitude of the cardiovascular response over time (from an original response of 18.4 ± 1.8 to 10.7 ± 3.0 mmHg at 5 min to 6.0 ± 1.9 mmHg at 10 min after the original injection; n = 5). Therefore, experiments were done by stimulating the VTA with glutamate at 20-min intervals because the magnitude of the response is not attenuated using these temporal parameters (24.1 ± 2.8, 23.6 ± 3.4, and 20.1 ± 3.6 mmHg for 3 consecutive microinjections at 20-min intervals; n = 11).

Experimental protocol. Cardiovascular responses to glutamate microinjections into the VTA were retested after administration of a 4% lidocaine saline solution in regions of ascending fiber bundles from the VTA. Glass micropipettes (50- to 75-µm tip) were used to microinject lidocaine as described before (Astra, Mississauga, Ontario) at a volume of 1 µl bilaterally in the medial forebrain bundle (3.7 posterior to bregma; 2.0 lateral to the midline; 8.5 ventral to the dura; pipette angled 10° in the anterior direction), periventricular fibers (4.3 posterior to bregma; 0.5 lateral to the midline; 6.0 ventral to the dura; angled 10° in the anterior direction), and fibers descending to the PAG (8.0 posterior to bregma; 0.5 lateral to the midline; 5.0 ventral to the dura; angled 6° in the posterior direction). The following protocol was used: 1) a site in the VTA that produced a >15-mmHg depressor response was identified; 2) 15 min later, lidocaine was injected contralaterally into the medial forebrain bundle, periventricular fiber system, or PAG; 3) 5 min after the first injection of lidocaine, the micropipette was slowly removed and an injection of lidocaine was done using the same micropipette in one of the ascending fiber systems ipsilateral to the VTA stimulation site; 4) the VTA site was restimulated at 5, 25, and 45 min after the final lidocaine injection. Experiments were also done in which a 10 mM solution of cobalt chloride or physiological saline were administered bilaterally in the PAG using the same protocol as described above. Only one experiment was done in each animal, which was followed by transcardial perfusion of 100 ml saline followed by 200 ml of 10% Formalin saline solution. Sections of the brain were cut on a cryostat and stained with thionin to verify the placements of the micropipettes in the VTA and fiber projections.

The effects of administration of lidocaine or cobalt chloride on the depressor responses were analyzed using ANOVA. Post hoc analysis using Tukey’s multiple comparison test was used to compare specific means when the ANOVA was found significant. A P value of <0.05 was taken to indicate statistical significance. Values are expressed as the means ± SE.

Anatomical experiments. Experiments were done in 17 male Sprague-Dawley rats (300–450 g) anesthetized with equithesin. The rats were placed in a Narishige stereotaxic frame, and a 1-mm burr hole was made above the midbrain region. A fine glass micropipette with a 15- to 25-µm tip containing a 5% solution of 10,000-MW lysine-fixable BDA (Sigma) dissolved into PBS was used to iontophoretically apply the tracer (4 µA anodal current for 15–30 min, 7s on/7s off) into the VTA. After a survival period of 5–12 days, rats were perfused transcardially with saline (0.9% NaCl, 200–300 ml) followed by 750 ml of a fixative containing 4% paraformaldehyde and 0.25% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The brain was removed and postfixed for 1 h in the same fixative. Coronal sections (75 µm) of the brain stem and midbrain were cut using a vibrating microtome, and sections were collected in PBS (0.1 M, pH 7.4) and washed (3 × 10 min) in PBS. The sections were then incubated in avidin-biotin-peroxidase complex (Vectastain ABC Elite, Vector Laboratories, Burlingame, CA) PBS solution containing 0.1% Triton X-100 for 4–6 h. Sections were rinsed in PBS (3 × 10 min) and Tris-buffer saline (TBS, 0.05 M, pH 7.6, 1 × 10 min), and reacted in TBS containing diaminobenzidine, hydrogen peroxide, and nickel ammonium sulfate (substrate kit for peroxidase, Vector Laboratories) followed by several rinses in PBS (4 × 10 min). Sections were mounted on gelatin-coated slides, allowed to dry overnight, dehydrated in alcohol, cleared in xylene, and coverslipped without staining.

Sections of the midbrain and brain stem were examined for BDA-labeled cells and fibers. Histochemistry with the ABC Elite kit provided sufficient background staining to delineate nuclei. Camera lucida drawings were made of representative
injections of BDA into the VTA and the BDA labeling in the brain stem. The photomicrographs were acquired by a light microscope (Zeiss, Germany) equipped with a digital camera (Quantix System, Photometrics, Tuscon, AZ) and viewed on a computer screen by using the program V for Windows (Quantix System) and printed using an ink jet printer.

RESULTS

Effect of Neuronal Block of Ascending Projections

Stimulation of the VTA (Fig. 1A) with glutamate (n = 11) elicited depressor and bradycardic responses that ranged in magnitudes of −15 to −30 mmHg (mean = −24.2 ± 1.8 mmHg) and −8 to −20 beats/min (mean = −12.8 ± 1.2 beats/min). Bilateral administrations of 1 µl of 4% lidocaine solution into the medial forebrain bundle and periventricular fiber system (Fig. 1A) had no effect on MAP or heart rate. The depressor responses elicited by glutamate stimulation of the VTA were not attenuated by the administration of lidocaine in ascending fiber systems (Fig. 1B). There were no significant differences in the level of MAP and heart rate during baseline (96.9 ± 3.3 mmHg and 330.4 ± 13.9 beats/min) and at 5 min (98.8 ± 3.0 mmHg and 309.9 ± 12.6 beats/min) and 25 min (99.5 ± 3.1 mmHg and 319.3 ± 8.2 beats/min) postlidocaine administration, indicating that the level of anesthesia was the same during the different test periods.

Anterograde Tracing of Efferents to Cardiovascular Centers in the Brain Stem

In 6 of the 17 animals receiving an injection of BDA, the spread of the tracer was confined to parts of the ventral mesencephalon that were shown in the preceding experiments (Fig. 1A) to produce cardiovascular depressor responses [also, see Kirouac and Ciriello (20) for detailed map of cardiovascular responsive sites]. Neuronal cell bodies that incorporated the BDA intracellularly were found in the lateral VTA, the transitional region between the VTA and the medial part of the substantia nigra pars compacta, and in the reticular formation above the medial lemniscus. The mammillary body and lateral hypothalamic area anterior to the injection site were not involved. The BDA injections also did not involve the red nucleus and the interpeduncular nucleus immediately caudal to the VTA. Labeled axons were seen to extend rostrally into the medial forebrain bundle and into the periventricular area as well as laterally through the substantia nigra pars compacta, and in the reticular formation above the medial lemniscus. The mammillary body and lateral hypothalamic area anterior to the injection site were not involved. The BDA injections also did not involve the red nucleus and the interpeduncular nucleus immediately caudal to the VTA. Labeled axons were seen to extend rostrally into the medial forebrain bundle and into the periventricular area as well as laterally through the substantia nigra (Fig. 2, A and B). Fibers leaving the injection site also traveled in a dorsocaudal direction throughout the midbrain tegmentum with the greatest density found in the midline and ipsilateral side of the BDA injection (Fig. 2C). Terminal labeling was found amongst the fibers in the pontine reticular nucleus, median raphe nucleus, and deep mesencephalic nucleus. A very dense plexus of
fiber terminals was found bilaterally in the ventrolateral PAG (PAGvl) and in the lateral portion of the dorsal raphe nucleus (Figs. 2, C and D, and 3). The terminals were a combination of fiber terminals and en passant varicose swellings. Light labeling was also found in the lateral and dorsal regions of the PAG. Fiber terminal labeling was seen in the rostrocaudal extent of the PAG with the highest concentration of BDA-labeled fibers and terminals being located in the caudal half of the PAG (Fig. 3C). Other regions of the brain stem associated with cardiovascular regulation were not labeled or were very weakly labeled (Fig. 2, E and F). The nucleus of the solitary tract and the A5 noradrenergic group did not show any BDA labeling after injections of BDA in the VTA. An occasional fiber with terminal boutons or varicose swelling were seen in the rostral and caudal ventrolateral medulla, raphe magnus nucleus, locus ceruleus, and parabrachial nucleus on the ipsilateral side of the injection (Fig. 2, E and F). As previously described in detail (4, 28), some regions not associated with regulation of the cardiovascular system were found to receive a weak to moderate projection from the VTA including the inferior olive, facial nucleus, and the medullary and pontine reticular nuclei.

Effect of Neuronal Block of Projections to the PAG

Bilateral injections of the neuronal blocking agent lidocaine in the PAG produced no cardiovascular responses, however, they resulted in a significant attenuation of the arterial pressure depressor responses elicited by stimulation of the VTA (F_{3,12} = 3.95, P < 0.04; Fig. 4B). The baseline response to VTA stimulation was attenuated by 51% at 5 min (P < 0.05, n = 4) and by 48% at 25 min (P < 0.05, n = 4) postlidocaine administration. Restimulation of the same site in the VTA at 45 min postlidocaine resulted in depressor response of similar magnitude as the baseline response (Figs. 4B and 5A). A similar pattern was observed for the heart rate responses, but it did not reach significance levels (Fig. 4B). The sites of the lidocaine injections were verified on histological sections to be located in the caudal portion of the PAG (Fig. 4A).

Bilateral microinjections of cobalt chloride also resulted in a significant attenuation of the arterial pressure depressor responses (F_{3,16} = 4.58, P < 0.02; Fig. 4B) from a baseline response of −26.9 ± 2.0 to −13.9 ± 3.4 mmHg at 5 min (P < 0.05, n = 5). Restimulation of the same site in the VTA at 25 and 45 min postcobalt chloride injection resulted in responses of the same magnitude (−19.4 ± 3.5 and −23.8 ± 0.9 mmHg, respectively) as the baseline responses (Figs. 4B and 5B). The effect of injections of cobalt chloride on the magnitude of the heart rate responses to VTA stimulation was not significant (Fig. 3B). Control injections of saline in the PAG had no effect on the cardiovascular depressor responses elicited from repeated stimulation of the same site in the VTA (n = 5; Fig. 4B). There were no differences in the MAP and heart rate levels for the lidocaine experiments during the baseline period (97.1 ± 2.9 mmHg and 344 ± 24.2 beats/min) and at 5 min (98.8 ± 2.2 mmHg and 336 ± 31.1 beats/min) and at 25 min (103.2 ± 2.7 mmHg and 346.7 ± 32.4 beats/min) after lidocaine administration. Similar to the lidocaine experiments, there were no differences in the baseline levels of arterial pressure and heart rate for the different times for the cobalt chloride and saline experiments (data not shown). There were no qualitative differences in the location of the lidocaine, cobalt

Fig. 3. Photomicrographs showing an example of an injection of BDA in the ventral mesencephalon (A) and the anterograde labeling seen in the periaqueductal gray matter (PAG; B and C). A: BDA injection site involving the lateral VTA and extreme medial aspect of the SN at the medial tip of the ml. The BDA injection site corresponds to the areas at which depressor responses were elicited by glutamate stimulation. B: low magnification of the PAG showing the dense bilateral fiber projection from injection site in A that innervates the PAG and dorsal raphe nucleus (DR). Note the dense cluster of fibers at the boundary region between the DR and the PAGvl (arrow) and moderately dense fiber projections to regions around the dense cluster. In some cases, weak labeling could be detected at higher magnification in the dorsolateral PAG (PAGdl). C: high magnification of the same area shown in B showing the high density of fibers and terminals in the region.
chloride, or saline injections in the PAG (Fig. 4A). The location of the lidocaine and cobalt chloride injections that attenuated the depressor responses elicited from stimulation of the VTA were in the same location in the caudal portion of the PAGvl that receives a dense projection from the VTA (Fig. 2).

DISCUSSION

This study suggests the existence of a cardiovascular depressor pathway from the VTA to the PAG. This conclusion is based on the finding of a dense bilateral projection from the VTA to the caudal aspect of the PAGvl. Blockade of neuronal impulses to the PAG with lidocaine attenuated the cardiovascular responses elicited by glutamate stimulation of the VTA. Moreover, blockade of synaptic transmission in the PAG with cobalt chloride also attenuated the cardiovascular responses from stimulation of the VTA, indicating that the response is at least partially mediated by fibers terminating in the PAG region. These findings are supported by the observations in the present investigation of an absence of descending projections from the VTA to other cardiovascular depressor centers in the brain stem. Neuronal block of ascending impulses to the forebrain and thalamus by administration of lidocaine into the medial forebrain bundle and periventricular fiber system failed to cause an attenuation of the depressor responses, indicating that a descending pathway from the VTA mediates the cardiovascular responses.

The neural pathways mediating the depressor responses from stimulation of the VTA were studied in the present investigation by the application of lidocaine or cobalt chloride in ascending and descending fiber projections from the VTA. Local injections of lidocaine and cobalt chloride have been used in previous studies to determine the functional anatomy of pathways mediating physiological functions (22). The local anesthetic
lidocaine prevents the propagation of nerve impulses by blocking voltage-sensitive sodium channels on cell bodies and fibers of passage (22, 26). Cobalt chloride prevents synaptic transmission without blocking conduction of fibers passing through the area by interfering with the function of calcium channels to prevent the release of synaptic transmitters (15, 22). The major advantages of using these substances are that they act in a reversible manner and that their actions are of suitable onset latency and duration to test for their effects after localized injections in potential pathways or terminal fields (22).

We found that bilateral injections of lidocaine into the PAG attenuate the cardiovascular responses to stimulation of the VTA. This effect was seen at 5 and 25 min postlido- cane injections and had dissipated at 45 min after the injection of lidocaine. The volume (1 µl) and concentration (4%) of lidocaine used was to produce maximal blockade of transmission to as much of the PAGvl as possible. Unilateral injections as well as smaller volumes and lower concentrations were ineffective in producing an attenuation in the magnitude of the depressor response (data not shown). We injected cobalt chloride to eliminate the possibility that the attenuation of the response by lidocaine was mediated by fibers passing through but not terminating in the PAG. Cobalt chloride effectively attenuated the response at 5 min postcobalt chloride injection, and the synaptic blockade had dissipated within 25 min. As with the lidocaine experiments, cobalt chloride only eliminated a portion of the depressor responses, suggesting that the lidocaine and cobalt chloride injections effectively diffused and blocked only a portion of the PAG. It is also possible that part of the cardiovascular depressor response is mediated by projections to regions near the PAG or to other unknown regions in the brain. This hypothesis is unlikely as regions such as the dorsal raphe nucleus or the lateral and dorsal regions of the PAG are associated with pressor and not depressor responses (2, 3, 8).

In addition, anterograde tracing experiments with the sensitive tracer BDA did not show a consistent projection to other depressor regions in the brain stem (caudal ventrolateral medulla, A5 area, nucleus of the solitary tract, ventromedial medulla, nucleus raphe magnus). It is also important to note that the administration of blocking agents to the PAG did not have an effect on arterial pressure or heart rate, indicating that the VTA does not tonically regulate the function of depressor neurons in the PAGvl.

Microinjections of lidocaine in the medial forebrain bundle, which contains the fibers of neurons of the VTA that innervate many regions of the forebrain (4, 23, 28), failed to attenuate the depressor response from stimulation of the VTA. Similarly, injections of lidocaine in the periventricular fiber system that contains the fibers of VTA neurons that innervate the thalamus and habenular complex (4, 23, 28) also failed to attenuate the depressor response. The results of these experiments indicate the VTA depressor responses are mediated by descending projections to brain stem or midbrain. We confirmed, using BDA as an anterograde tracer, the results of previous anatomical studies (4, 23, 28) showing the lack of descending projections from the VTA to the cardiovascular centers in the brain stem. Injections of BDA that were confined to regions in the ventral mesencephalon that are capable of producing cardiovascular depressor responses (lateral VTA, medial substantia nigra, and regions immediately dorsal to the medial lemniscus) (20) produced no labeling in the nucleus of the solitary tract and the A5 catecholaminergic cell group and only an occasional fiber and few terminals in the ventrolateral and ventromedial medulla and medial raphe nucleus. However, a very dense bilateral projection was found to innervate the PAGvl and the lateral wing of the dorsal raphe nucleus, regions that have been strongly implicated in cardiovascular control (2, 3, 8, 13, 31).

Previous anatomical studies done in the late 1970s using anterograde transport of tritiated amino acids showed that the PAG received a heavy projection from the VTA (4, 28). However, the magnitude of this projection was not apparent from these studies, and tracing of projections with tritiated amino acid does not allow investigators to make the distinction between axons and fiber terminals. The present study done using BDA, a new and very sensitive anterograde tracer (14, 33), demonstrates a dense VTA projection terminating in the PAGvl. Neurons in the VTA were also labeled after injections of the retrograde tracing substance horseradish peroxidase confirming the existence of a VTA-PAGvl projection (6). As shown in the present investigation, the VTA projects strongly to the rostrocaudal extent of the PAGvl with the densest projection being located in the caudal half of the PAGvl. Therefore, the results of the tract-tracing experiments along with the lidocaine and cobalt chloride experiments strongly support the hypothesis that a VTA-PAGvl pathway mediates the depressor responses elicited from the VTA.

A large amount of literature has accumulated on the role of the PAG in regulating emotional expression and cardiovascular function (2, 3, 5, 8, 13, 31). Stimulation of the dorsal and lateral PAG elicits defensive behaviors, hypertension, and tachycardia, whereas stimulation of the PAGvl produces hypoactivity, hypotension, and bradycardia (2, 3, 8). The ventrolateral region of the caudal PAG contains longitudinal columns of neurons that regulate the cardiovascular system (2, 3, 8, 16, 21). Stimulation of the PAGvl elicits cardiovascular depressor responses as well as analgesia and behavioral immobility in awake animals (5, 8, 16, 18, 21). The depressor responses elicited from stimulation of the PAGvl are mediated by injections to depressor regions in the caudal midline and ventrolateral medulla (9, 16). The depressor regions of the caudal medulla act via an ascending GABAergic projection to vasomotor neurons in the rostral ventrolateral medulla that control the level of sympathetic nerve activity to the cardiovascular system (13, 31). Therefore, activation of a VTA-PAGvl pathway may result in depressor responses by activation of GABAergic neurons in the caudal ventrolateral
medulla that, in turn, act to inhibit vasomotor neurons in the rostral ventrolateral medulla.

Perspectives

There is increasing evidence for a role for the VTA in cardiovascular regulation. For example, chemical stimulation of discrete regions of the VTA with microinjections of glutamate produces cardiovascular depressor responses mediated by a decrease in sympathetic nerve activity to the cardiovascular system (20). In contrast, others have reported that activation of neurons in the VTA with relatively large injections of a neuropeptide agonist elicited hypertensive responses that are mediated by an increase in plasma vasopressin and inhibition of the baroreflexes (10, 11, 12). The depressor responses elicited by stimulation of the VTA appear to involve activation of dopamine D2 receptors in the brain as pretreatment with intravascular administration of the D2 dopamine antagonist raclopride attenuated the depressor responses from stimulation of the VTA (20). Dopamine is a possible candidate for the neurotransmitter mediating the response because large portions of neurons in the VTA are dopaminergic (23). A low to moderate number of dopamine D2 receptors have also been demonstrated in the PAG using the sensitive in situ hybridization method (34). However, to our knowledge, there is no evidence in the literature that we are aware of showing that dopaminergic neurons in the VTA project to the PAG or that dopamine administrations in the PAG produced cardiovascular responses. There is also some evidence that glutamate, cholecystokinin, and neurotensin are colocalized with dopamine in VTA neurons, and these neurotransmitters could mediate the effects seen by stimulation of the VTA (17, 27, 30). Future experiments will attempt to determine the neurotransmitters and receptors involved in the VTA-PAG depressor pathway.

The PAG has been implicated in a variety of integrative functions including analgesia, autonomic regulation, fear and rage reactions, lordosis, and others (3, 5). This suggests that the analgesic, autonomic, and behavioral reactions evoked by neuronal circuitry in the PAG represent coordinated responses that play a role in an organism's survival. It is also well known that dopaminergic neurons in the VTA are strongly activated by behaviorally relevant stimuli (23). However, speculative at this time, a VTA-PAG projection may be important in the regulation of neuronal circuitry of the PAG involved in the coordination of physiological and behavioral responses. This may include cardiovascular responses that are involved in the behavioral reactivity to noxious or stressful stimuli. It will also be important to determine if activation of the VTA modulates the behavioral and cardiovascular components of the defense reaction.

In summary, the present investigation shows that depressor regions of the PAGvl receive a strong projection from the VTA. Descending projections to other cardiovascular regulatory centers in the brain stem were absent or very weak after large injections of BDA in the VTA. Blockade of the neuronal transmission with lidocaine or blockade of synaptic transmission with cobalt chloride in the PAGvl region attenuated the cardiovascular depressor responses produced by stimulation of the VTA. In contrast, blockade of neuronal transmission in ascending pathways had no effect on the depressor responses. The results of these experiments strongly suggest the existence of a VTA-PAGvl cardiovascular depressor pathway.

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