Sympathoinhibition evoked from caudal midline medulla is mediated by GABA receptors in rostral VLM

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Coleman, Matthew J., and Roger A. L. Dampney. Sympathoinhibition evoked from caudal midline medulla is mediated by GABA receptors in rostral VLM. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R318–R323, 1998.—The present study was performed to determine whether the powerful depressor and sympathoinhibitory response that can be evoked from neurons in the caudal midline medulla is mediated by γ-aminobutyric acidergic (GABAergic) inhibition of sympathoexcitatory neurons in the rostral part of the ventrolateral medulla (VLM). In anesthetized baroaract and barodenervated rabbits, bilateral microinjections of bicuculline into sympathoexcitatory sites in the rostral VLM resulted in a sustained increase in renal sympathetic nerve activity and abolished or reversed the depressor and sympathoinhibitory response evoked by glutamate microinjection into the caudal midline medulla. By contrast, the sympathoinhibitory response evoked from the caudal midline medulla persisted when the background level of renal sympathetic nerve activity was reflexly raised by baroreceptor unloading. The results indicate that 1) the depressor and sympathoinhibitory response evoked by stimulation of neurons in the caudal midline medulla is mediated by a GABAergic synapse in the rostral VLM and 2) there are also sympathoexcitatory neurons in the caudal midline medulla whose presence is revealed by blockade of the more powerful sympathoinhibitory response.

γ-aminobutyric acid; ventrolateral medulla; baroreceptor reflex; raphe pallidus and obscurus; renal sympathetic nerve activity

It is well established that neurons within the midline raphe nuclei in the medulla can influence the sympathetic outflow and blood pressure. In particular, many studies have shown that electrical or chemical stimulation of sites within the rostral medullary raphe nuclei (i.e., from the level of the obex to the pontomedullary border) elicits either an increase or decrease in blood pressure and sympathetic activity, depending on the specific site of stimulation (1, 6, 13, 18, 19, 22, 23, 30). These variable patterns of cardiovascular responses presumably reflect the fact that the medullary raphe nuclei contain both sympathoexcitatory and sympathoinhibitory neurons, as indicated by single cell recording experiments (20, 24).

Recently, we have shown that a powerful depressor and sympathoinhibitory response is produced by stimulation of neurons in a discrete part of the raphe obscurus and pallidus in the caudal midline medulla of the rabbit (8). These neurons are a separate population from the sympathoinhibitory neurons in the caudal part of the ventrolateral medulla (VLM) (5). Furthermore, they also appear to be a separate group from the previously described sympathoinhibitory neurons in the rostral medullary raphe nuclei (19, 20, 22, 25) because, apart from their much more caudal location, they do not appear to exert a tonic inhibitory effect on sympathetic activity and blood pressure, unlike the sympathoinhibitory neurons in the rostral medullary raphe (19).

It is most unlikely that the sympathoinhibitory effects elicited by stimulation of the caudal medullary medullary neurons in the rabbit are mediated by a direct descending projection to sympathetic preganglionic neurons, because anatomical studies in this species have demonstrated that there are very few spinal projecting neurons in this part of the medulla (4, 16). This raises the possibility that the pathway mediating this response includes an inhibitory synapse with bulbospinal sympathoexcitatory neurons in the rostral part of the VLM, because the latter group of neurons has been shown to be a site of convergence of both excitatory and inhibitory inputs arising from many different sources (for review, see Ref. 9). In particular, it has been shown that sympathoexcitatory neurons in the rostral medullary raphe (19) as well as those in the caudal VLM (3) inhibit rostral VLM neurons via a GABAergic synapse.

METHODS

General procedures. Experiments were performed on 13 New Zealand White rabbits (2.1–3.7 kg body wt) of either sex. A marginal ear vein was cannulated, and the rabbits were anesthetized with pentobarbital sodium (35 mg/kg iv initially, followed by continuous iv infusion of 9–15 mg·kg−1·h−1). Body temperature was monitored with a rectal probe and maintained in the range 38–39°C with a thermoregulated heating lamp. The trachea was cannulated, and catheters were placed in a femoral artery and a femoral vein. When a stable and adequate level of anesthesia was achieved, the dorsal medulla was exposed according to the procedure described previously (27). In two rabbits, the arterial baroreceptors were denervated (see Baroreceptor denervation) before exposure of the dorsal medulla. The rabbits were artificially ventilated with oxygen-enriched air at a level that maintained end-tidal CO2 close to 4% and were paralyzed with alcuronium chloride (0.1–0.2 mg/kg iv every 2–3 h). The effects of the alcuronium chloride were allowed to wear off before each additional dose was administered. The adequacy of anesthesia without paralysis was verified by the absence of a withdrawal response to nociceptive stimulation of a hindpaw, and during paralysis, by a stable arterial pressure, sympathetic nerve activity, and heart rate. Arterial pressure was measured via the femoral arterial catheter, and the mean
Baroreceptor denervation. In two experiments, the carotid sinus bifurcations were denervated as previously described (27) and the aortic and vagal nerves were cut to eliminate baroreceptor reflex effects that may have been secondarily evoked, as a consequence of the depressor response elicited from the caudal midline medulla. The effectiveness of carotid denervation was confirmed by the absence of a reflex response to carotid occlusion.

Renal nerve recording. After exposure of the renal nerve, its distal end was cut or crushed to eliminate afferent discharge and its proximal end was placed on bipolar silver recording electrodes and covered with mineral oil. The signal from the electrodes was amplified, passed through a band-pass filter (100–1,000 Hz), displayed on a cathode ray oscilloscope, and monitored by means of an audio amplifier. The filtered nerve activity signal was recorded on magnetic tape and was also rectified and integrated (resetting every 5 s). The integrated activity was displayed on a polygon chart recorder. At the end of the experiment, the baseline noise level was established by applying Xylocaine to the renal nerve proximal to the recording electrodes.

Intramedullary microinjections. Microinjections of sodium glutamate (20 or 200 mM) and bicuculline (4 or 20 mM) were made into medullary sites using a micropipette held in a micromanipulator. In all cases, the vehicle solution was 20 mM phosphate-buffered saline. The volume was 20 nl for glutamate microinjections and 50–100 nl for the bicuculline microinjections. The injectate contained either 0.015% wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP) or a low concentration of rhodamine or fluorescein isothiocyanate (FITC)-labeled microspheres to enable later histological verification of the injection sites. The rostrocaudal, mediolateral, and dorsoventral coordinates of the micropipette tip were determined with respect to the obex, midline, and dorsal surface, respectively. Injections were made by pressure, as previously described (27), and the volume injected was measured by determining the displacement of the meniscus in the pipette with respect to a horizontal grid viewed through an operating microscope.

Experimental procedure. Microinjections of glutamate were first made into the caudal midline medulla to identify a site at which a distinct depressor and sympathoinhibitory response was evoked. Usually fewer than three penetrations of the medulla were required to identify such a site. Microinjections of glutamate were then made into the rostral VLM to localize the pressor region. Two or three penetrations were made into the rostral VLM, and glutamate microinjections were made at different depths. The coordinates of the sites at which microinjection of <5 nmol of glutamate evoked distinct pressor responses with short onset latency (<5 s) were noted. The glutamate micropipette was then withdrawn and replaced with another micropipette containing bicuculline. Microinjections of bicuculline were then made into the pressor sites as identified by glutamate microinjection. In two experiments in which a distinct short-latency pressor response was elicited by glutamate stimulation at only a single site in the rostral VLM, a single microinjection of bicuculline (200 pmol) was made into this site. In all other experiments, between two and four microinjections were made into different sites (0.5 mm apart) in the rostral VLM, depending on the extent of the pressor region as indicated by glutamate stimulation. The same procedure was then repeated on the other side of the medulla. In five rabbits in which the lower concentration of bicuculline (4 mM) was injected, the total amount of bicuculline injected into the rostral VLM was 200–1,000 pmol per side, and in the remaining rabbits in which the higher concentration of bicuculline (20 mM) was injected, the total amount injected was 4–8 nmol per side.

After a waiting period after the bicuculline injections varying between 6 and 67 min (depending on the time until the resting arterial pressure and sympathetic nerve activity had stabilized), another glutamate microinjection was made into the original depressor and sympathoinhibitory site in the caudal midline medulla. Further injections were then made into surrounding sites, separated from each other by 0.5 mm in the dorsorostral or rostrocaudal direction.

Statistical analysis. Comparisons between responses before and after injection of bicuculline into the rostral VLM were made using the paired t-test. A P value of <0.05 was taken to indicate a statistically significant difference. All values are expressed as means ± SE.

RESULTS

Effects of bilateral injections of bicuculline into the rostral VLM pressor region on the response evoked from the caudal midline medulla. In agreement with our previous study (8), microinjections of glutamate into a restricted region within the caudal midline medulla evoked a marked decrease of 20 ± 2 mmHg in mean arterial pressure (MAP) (P < 0.001) and of 77 ± 5% in renal sympathetic nerve activity (RSNA) (P < 0.001) compared with control values in eight baroindact rabbits. An example of a typical response is shown in Fig. 1A. A similar pattern of response was also observed in two baroindact rabbits (decreases of 24 and 20 mmHg in MAP and of 81 and 32% of control in RSNA).

After bilateral microinjection of bicuculline into the rostral VLM pressor region, MAP increased rapidly (maximum increase of 52 ± 7 mmHg, P < 0.001) and then gradually declined to a new resting level of 106 ± 6 mmHg, which was not significantly different (P > 0.3) from the precuculline resting level of 97 ± 3 mmHg. The RSNA also increased rapidly [to a level of 313 ± 58% (P < 0.01) of the precuculline value at the point where the increase in MAP was maximal] but then remained close to this elevated level while MAP gradually declined. After a waiting period of 6–67 min after the bicuculline injection, when MAP and RSNA had
stabilized, a glutamate injection was made into the same site in the caudal midline medulla at which a depressor and sympathoinhibitory response had been evoked initially. In three of the eight barointact rabbits and in both barodenervated rabbits, the depressor and sympathoinhibitory response was abolished. Additional injections of glutamate were also made into sites surrounding the initial depressor site, and in all cases, no response was elicited from any surrounding site.

In the remaining five rabbits, the depressor and sympathoinhibitory response to glutamate microinjection into the caudal midline medulla was reversed to a pressor and sympathoexcitatory response after bicuculline injection into the rostral VLM pressor region, with an increase of 11 ± 2 mmHg (P < 0.01) in MAP and 52 ± 14% (P < 0.05) in RSNA. An example of such a response is shown in Fig. 1B. The reversal of the sympathoinhibitory response to a sympathoexcitatory response was not dependent on the dose of bicuculline, because in three cases it occurred when the lower concentration (4 mM) of bicuculline was injected and in two cases, when the higher concentration (20 mM) was injected. Similarly, the reversal was not dependent on the waiting time after the bicuculline injection, because it occurred at both relatively short (<10 min) and longer (>40 min) times after the bicuculline injection.

Histological examination showed that the postbicuculline glutamate injection sites (marked with FITC-labeled microspheres) in the caudal midline medulla were either overlapping with or close to (within 0.5–1.0 mm) the prebicuculline glutamate injection sites (marked with rhodamine-labeled microspheres).

Effects of raising the background level of sympathetic activity on the response evoked from the caudal midline medulla. As mentioned above, bicuculline injection into the rostral VLM resulted in an increase in the resting level of MAP and RSNA. Three experiments were therefore performed to test the possibility that an increase in the background level of sympathetic nerve activity may have affected the response to stimulation of the caudal midline medulla.

In these three experiments, all of which were performed in barointact animals, glutamate injection resulted in a decrease of 26 ± 7 mmHg in MAP and 67 ± 17% in RSNA (Fig. 2A). After the MAP and RSNA had stabilized again after this response, sodium nitroprusside (0.1–0.15 mg/kg) was injected intravenously, resulting in a decrease in MAP of ~40 mmHg and a reflex increase in RSNA to 208 ± 33% of the resting value (Fig. 2B). At this point, while the RSNA was elevated, a glutamate injection into the depressor site in the caudal midline medulla was repeated (Fig. 2B). In all three animals, this injection resulted in a large decrease in RSNA (mean decrease of 51 ± 17% from the new increased baseline level), which was not significantly different (P > 0.2) from the responses elicited before injection of sodium nitroprusside.

**DISCUSSION**

The results show clearly that the marked depressor and sympathoinhibitory effects that are elicited by stimulation of cell bodies in the caudal midline medulla were abolished, and in some cases reversed, by bilateral injection of bicuculline into the rostral VLM. This finding therefore indicates that the depressor and sympathoinhibitory response from the caudal midline medulla...
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The sympathetic activity remained elevated during the waiting period after the bicuculline injection, indicating that the block of GABAergic inputs to rostral VLM sympathoexcitatory neurons was sustained. It could therefore be argued that the abolition of the sympathoinhibitory response to stimulation of the caudal midline medulla was a consequence of the raised level of the background sympathetic nerve activity rather than being due specifically to the block of GABAergic synapses in the rostral VLM. This seems unlikely, however, because powerful sympathoinhibition could still be elicited in those animals whose background level of sympathetic nerve activity was markedly increased by unloading of arterial baroreceptors. Furthermore, these control experiments also demonstrated that sympathoinhibitory responses could be elicited by a second glutamate injection into the caudal midline medulla, ruling out the possibility that the abolition of the response was simply due to the refractoriness of the sympathoinhibitory neurons in the caudal midline medulla to a repeated stimulation. This is further supported by our previous study in which we showed that sympathoinhibitory responses were evoked by repeated injections into the same site (8).

Apart from its effects on bulbospinal sympathoexcitatory neurons, bicuculline may also elicit an increase in the activity of other neurons in the rostral VLM. Conceivably, such neurons could project back to the sympathoinhibitory neurons in the caudal midline medulla and act to inhibit their responsiveness to glutamate stimulation. A recent retrograde tracing study in our laboratory, however, did not reveal a projection to the caudal midline medulla from the rostral VLM or from other brain stem regions that might relay signals from the rostral VLM (Coleman and Dampney, unpublished observations). Thus it seems most likely that the abolition of the sympathoinhibitory response evoked from the caudal midline medulla was due to blockade of GABAergic inputs to sympathoexcitatory neurons in the rostral VLM rather than to indirect effects involving neurons in regions outside the rostral VLM.

A previous electrophysiological study in the cat showed that electrical stimulation of sites in the midline medulla elicited a sympathoinhibitory response and inhibition of sympathoexcitatory neurons in the rostral VLM (19). Furthermore, the inhibition of the rostral VLM sympathoexcitatory neurons was blocked by iontophoretic application of bicuculline, indicating that the sympathoinhibitory response evoked from the midline medulla in that study was, as in the present study, mediated by a GABAergic synapse on sympathoexcitatory neurons in the rostral VLM. There are, however, two important lines of evidence that indicate that the sympathoinhibitory neurons in the medullary midline of the cat, as described by McCall (19), are not the same group as those that we have identified in this and a previous study in the rabbit (8). First, the sites in the midline of the cat that produce depressor and sympathoinhibitory responses are located between 1 and 6 mm rostral to the obex (19), whereas those in the rabbit are much more caudal, located between the obex and 2 mm more caudal (8). Second, lesions of the rostral medullary midline in the cat result in an increase in sympathetic activity, indicating that the sympathoinhibitory neurons in this region are tonically active (21). By contrast, large multiple injections of GABA or muscimol into the caudal midline medulla of the rabbit have no effect on arterial pressure or renal sympathetic activity, indicating that the sympathoinhibitory neurons in this region have no tonic activity (8). Thus, taken together, this anatomical and functional evidence indicates that the sympathoinhibitory neurons in the caudal midline medulla of the rabbit are not homologous with the sympathoinhibitory neurons previously identified in the rostral midline medulla of the cat.

In half of the experiments, a pressor and sympathoexcitatory response was evoked from the caudal midline medulla after bicuculline injection into the rostral VLM. This observation demonstrates that there are also sympathoexcitatory neurons in this region. Consistent with this, a pressor and sympathoexcitatory re-
response is occasionally elicited from the caudal midline medulla even in animals in which GABAergic inhibition of rostral VLM neurons is not blocked (8). Thus the results of the present study support the view that the caudal midline medulla contains both sympathoexcitatory and sympathoinhibitory neurons, although the latter group appears to be predominant.

What is the pathway between the sympathoinhibitory neurons in the caudal midline medulla and the sympathoexcitatory neurons in the rostral VLM? It is unlikely that it is a direct pathway, because anatomical studies have shown that there are few neurons in the caudal midline medulla that project directly to the rostral VLM in the rabbit (26). It is possible that the pathway from the caudal midline medulla to the rostral VLM includes a synapse in the caudal VLM, because the latter region also contains neurons that inhibit sympathetic activity via a GABAergic synapse with sympathoexcitatory neurons in the rostral VLM (3, 29). Furthermore, neurons in the caudal VLM have been shown to act as interneurons in the central pathway, mediating the inhibition of rostral VLM sympathoexcitatory neurons elicited by stimulation of arterial baroreceptors or other peripheral receptors (2, 11, 12, 17, 28). Thus it is possible that neurons in the caudal VLM are a site of convergence of inputs from different sources that elicit sympathoinhibitory responses, including those originating from the caudal midline medulla. Further studies are needed, however, to determine this.

It is well known that sympathoexcitatory neurons in the rostral VLM receive a powerful tonic GABAergic inhibitory input, which originates from both peripheral baroreceptors as well as other sources that are independent of baroreceptors (for review, see Ref. 9). It is most unlikely, however, that the neurons in the caudal midline medulla contribute to this tonic inhibition, because they are themselves not tonically active at normal levels of blood pressure (8).

Perspectives

Our observations raise the important question as to the origin of the inputs to the sympathoinhibitory neurons in the caudal midline medulla and of the physiological conditions that lead to activation of these neurons. As mentioned above, it is unlikely that the sympathoinhibitory neurons receive arterial baroreceptor or other tonically active inputs. On the other hand, anatomical studies in the rat have shown that there are major projections to the raphe pallidus in the caudal midline medulla from the posterior hypothalamus (15) and the caudal ventrolateral part of the periaqueductal gray (14). The latter region is of particular interest because there is considerable evidence that it integrates depressor and sympathoinhibitory responses associated with certain types of behavior (7). Similarly, in the rabbit, we have found that there are projections to the depressor region in the caudal midline medulla from the hypothalamus and the caudal ventrolateral periaqueductal gray (Coleman and Dampney, unpublished observations). Thus the caudal midline medulla may play an important role in mediating depressor and sympathoinhibitory responses that are part of more complex behaviors integrated within midbrain and forebrain regions. In this regard, our observation that powerful sympathoinhibition could still be evoked from the caudal midline medulla even when the baroreceptors were unloaded is interesting, because it indicates that inhibition of presympathetic neurons in the rostral VLM by inputs originating from the caudal midline medulla is capable of overriding baroreceptor inputs. This is further indicated by the finding that the sympathoinhibition was abolished or reversed in all experiments, regardless of whether the baroreceptors were intact or denervated.

In conclusion, the results of this study demonstrate that the powerful depressor and sympathoinhibitory response that is evoked from the caudal midline medulla is mediated by a GABAergic synapse in the rostral VLM. There are also sympathoexcitatory neurons in the caudal midline medulla, whose presence was revealed by blockade of the more powerful sympathoinhibitory response. The sympathoexcitatory neurons in the caudal midline medulla do not appear to contribute to the tonic GABAergic inhibition of rostral VLM sympathoexcitatory neurons but may be part of the central pathways subserving depressor responses originating from higher centers. Further anatomical and physiological studies will be needed to test this hypothesis.

This work was supported by the National Health and Medical Research Council and the Ramaciotti Foundations of Australia.

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Received 11 April 1997; accepted in final form 14 October 1997.

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