Role of the Nucleus Ambiguus in the Regulation of Heart Rate and Arterial Pressure

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SUMMARY The present study examined the effect of lesion of cell bodies in the nucleus ambiguus area on the development of neurogenic hypertension and further explored the cardiovascular responses produced by chemical and electrical stimulation of the nucleus ambiguus and the neighboring C1 region. Three days after chemical lesion of the nucleus ambiguus with kainic acid, arterial pressure and heart rate were unchanged; however, subsequent sinoaortic deafferentation produced a significantly greater increase of arterial pressure (157 ± 7 vs 132 ± 5 mm Hg) and heart rate (436 ± 10 vs 374 ± 10 beats/min) compared with those produced by sham lesion. Glutamate injected into the nucleus ambiguus increased arterial pressure and heart rate at 20 nmol/100 nl and decreased heart rate at 50 nmol/100 nl. Glutamate injected into the C1 area increased arterial pressure and heart rate at both doses. γ-Aminobutyric acid at 50 nmol/100 nl produced bradycardia and a fall in arterial pressure when injected into both the nucleus ambiguus and C1 area. The heart rate responses to γ-aminobutyric acid and glutamate were attenuated in sinoaortic-deafferentated rats. The nucleus ambiguus and the C1 region were mapped using electrical stimulation with microelectrodes. All points stimulated in three anteroposterior sections in the nucleus ambiguus and the C1 area produced increases in arterial pressure, whereas bradycardia was restricted to the middle of three lateral coordinates associated with the center of the nucleus ambiguus and the C1 area ventral to the nucleus ambiguus. These data indicate that 1) chemical lesion of cell bodies of the nucleus ambiguus enhances the increase of arterial pressure after sinoaortic deafferentation and 2) electrical and chemical stimulation of the nucleus ambiguus area produce both bradycardia and increased arterial pressure. The results obtained with kainic acid lesions of the nucleus ambiguus suggest that this area exerts an inhibitory influence on sympathetic vasomotor tone that, when removed, could enhance the increase of arterial pressure after sinoaortic deafferentation. We conclude that the nucleus ambiguus is involved not only in heart rate control but also in central regulation of arterial pressure through pressor systems, and perhaps separately with neuronal projections associated with the baroreceptor reflex. (Hypertension 11: 602-607, 1988)

KEY WORDS • cardiovascular regulation • baroreceptor reflex • sinoaortic deafferentation • electrical stimulation • glutamate • γ-aminobutyric acid • kainic acid

In previous studies, we observed that electrolytic lesion of the nucleus ambiguous (NA) facilitates the development of hypertension in rats with sinoaortic deafferentation (SAD) and that electrical stimulation of the NA produces a bradycardic effect and an independent pressor response. These results suggested that the NA may be related to autonomic control not only of heart rate (HR) but of arterial pressure (AP) as well.

The NA, a vagal cardioinhibitory area, is an important component of circulatory adjustments to changes in AP, but its anatomical and physiological interrelationships to other autonomic control regions of the central nervous system are not completely understood. To discriminate the effect of lesion of fibers of passage from that of cell bodies, in the present study we used kainic acid (KA), a structural analogue of the amino acid neurotransmitter L-glutamate, to produce lesion of the cell bodies in the NA.

In our previous work, electrical stimulation of the NA area elicited bradycardia that was not reflex in origin since it was maintained in SAD rats. To characterize this area anatomically and functionally, it was...
mapped in the present study employing microelectrode stimulation. On the other hand, several investigators have indicated the presence of receptors for glutamate in pressor and depressor areas in brainstem and have suggested that L-glutamate is a candidate neurotransmitter released by baroreceptor afferent nerves. Recent work has suggested that glutamatergic transmitter is also involved at the level of ventrolateral medulla (VLM) in mediating or modulating both vagal and sympathetic baroreceptor reflexes. To help determine whether two distinct populations of pressor and cardioinhibitory neurons exist in the NA, chemical stimulation of this area with microinjection of glutamate was performed. The inhibitory agent γ-aminobutyric acid (GABA) was also injected to examine the effect of inhibitory mechanisms in the NA on HR and AP control.

Vasomotor tone is dependent in large part on tonically active neurons in the C1 area of VLM. Since the NA and the C1 region are juxtaposed anatomically, it was important for us to attempt to distinguish between the cardiovascular control exerted by these two areas of brainstem. Thus, experiments using chemical and electrical stimulation of the NA also examined the C1 area.

Materials and Methods

Male Sprague-Dawley rats (Biolab, St. Paul, MN, USA) weighing 300 to 350 g were used in these experiments. In experiments performed in conscious rats submitted to lesion of the NA and SAD, direct AP was measured by means of a polyethylene cannula (PE-10 connected to PE-50, Clay Adams, Parsippany, NJ, USA) inserted into the abdominal aorta through the femoral artery. Another cannula was inserted into femoral vein for administration of drugs. These procedures were performed with the rats under anesthesia with ketamine (120 mg/kg) and acepromazine maleate (12 mg/kg) 24 hours before the recording session. In this protocol, as well as in all others, the arterial catheter was connected to a Century CP-01 pressure transducer (Inglewood, CA, USA) and a Beckman recorder (Model R 611, Fullerton, CA, USA), and the HR was derived from the arterial pulse with a Beckman 9857B cardiotachometer.

Lesions of the NA with KA were made in two steps with the rats under ketamine plus acepromazine anesthesia. KA was first injected into the right NA and 2 days later into the left NA to avoid the respiratory problems that arise when the agent is injected bilaterally. KA (200 ng/100 nl) was injected through a glass micropipette with a tip diameter of 50 μm connected to a 1-μl Hamilton microsyringe (Reno, NV, USA). The micropipette was placed in the NA in accordance with the coordinates of Paxinos and Watson3 (−3.8 mm posterior and +0.4 mm dorsal referred to the interaural line and 2.1 mm lateral to midline).

SAD was performed according to the technique described by Krieger.4 The efficacy of deafferentation was tested by injection of phenylephrine (5 μg/kg); rats with bradycardia smaller than 24 beats/min were considered to have adequate SAD. In this protocol the rats underwent the SAD procedure 3 days after second injection of KA and were studied again 3 days later.

Glutamate was injected bilaterally into the NA and the C1 area in control and SAD rats under urethane anesthesia. Two doses, 20 and 50 nmol in 100 nl, were injected sequentially using a 33-gauge injector through a stereotaxically placed 23-gauge guide cannula. Between injections the guide cannula was cleaned with saline. After glutamate, GABA was injected (50 nmol in 100 nl). After injection of glutamate and GABA, the areas were electrolytically lesioned for later histological analysis.

The anatomical mapping of the NA area was performed in eight normotensive control rats (weight, 337 ± 6 g) under urethane anesthesia. Unilateral stimulation of the right NA was performed according to the coordinates of Paxinos and Watson.3 We stimulated points in three sections rostrocaudal (−3.6, −3.8, and −4.0 in relation to the interaural line), in three coordinates lateral to the midline (2.0, 2.1, and 2.2), and in five coordinates dorsoventral (+0.4, +0.2, 0.0, −0.2, −0.4 in relation to the interaural line). The rostrocaudal and lateral coordinates were selected randomly, but the sequence of stimulations in the dorsoventral plane was always made in the same track. The duration of each pulse was 0.5 msec, and the stimulus was maintained during 10 seconds, with the current fixed at 200 μA and frequency at 100 Hz. In these experiments, monopolar tungsten electrodes (tip diameter, 50 μm) were used and AP and HR were recorded. After the stimulation map was completed, the electrode was placed in the central point of the field stimulated (anteroposterior, −3.8; dorsoventral, 0.0; lateral, 2.1) and a small marking lesion was made using DC anodal current of 0.5 mA for 5 to 7 seconds delivered by a Grass stimulator (Quincy, MA, USA) through a Grass constant current unit.

At the end of all the experiments the rats were killed by intracardiac perfusion with saline followed by 10% buffered formalin. The brains were removed and stored in buffered formalin for 2 days, and serial frozen transverse sections at 40 μm were cut and stained using the cresyl violet method. The extent of the chemical lesions or the locus of marking lesions was mapped using comparisons of the sections with the stereotaxic atlas of Paxinos and Watson.3 An unpaired Student's t test was used to compare the groups, and a probability level below 0.05 was used to identify statistical significance.

Results

Lesions of the Nucleus Ambigous

Rats submitted to NA lesion with KA and studied 3 days later showed no significant changes in AP (110 ± 3 vs 116 ± 2 mm Hg) or HR (317 ± 15 vs 290 ± 7 beats/min) when compared with rats submitted to sham lesion with saline. The lesioned rats did, however, exhibit a significant reduction in the reflex bradycardic response to a pressor dose of phenylephrine (−68 ±
HR (500 bpm) and MAP were measured. These parameters were measured 3 days after SAD in rats with previous lesion of the nucleus ambiguus. Asterisk indicates significant difference (p < 0.05).

The same groups were submitted to SAD and studied 3 days later. As shown in Figure 1, rats with previous NA lesion produced with KA demonstrated a greater increase in mean arterial pressure (MAP) and HR when compared with the sham-lesioned group receiving SAD alone.

A summary of the sites of the KA-induced lesions is shown in Figure 2, which is a schematic of a coronal section of medulla 1.5 mm rostral to the obex summarizing the overlap analysis of sites of damage in the brainstem of five rats. The area of damage was characterized by gliosis and the loss of cell bodies. The extent of the damaged area varied but the lesion was centered in the NA.

Injection of Glutamate and γ-Aminobutyric Acid into the Nucleus Ambiguus and the C1 Area

Glutamate in two doses, 20 and 50 nmol in 100 nl, was injected bilaterally into the NA and the C1 area in control and SAD rats. As shown in Figure 3, glutamate injected into the NA increased MAP, but there was no significant difference between doses. In control rats, the lower dose of glutamate (20 nmol/100 nl) increased HR (+22 ± 5 beats) while the higher dose (50 nmol/100 nl) induced bradycardia (-26 ± 15 beats/min). Changes in HR were much smaller in SAD than in control rats.

Glutamate injected into the C1 area produced similar increases of MAP at both doses in control and SAD rats. In contrast to injections into the NA, glutamate injected into the C1 area increased HR at both doses. GABA administered to the NA and the C1 area induced similar reductions in MAP in both control and SAD rats, however, the fall in HR in SAD rats was attenuated. In four animals, treatment with propranolol, 1 mg/kg, abolished the bradycardia produced by injection of GABA into the NA region. Injection of saline into the NA in three rats produced insignificant changes in MAP and HR (3 ± 2 mm Hg and 4 ± 2 beats/min).

Mapping the Nucleus Ambiguus and the C1 Area

Figure 4 summarizes the results obtained with electrical stimulation of sections -3.6, -3.8, and -4.0. The locus of the major anatomical representation of the NA (-3.8) is summarized in Figure 4B. The 15 sites of stimulation are depicted in the coronal section on the left. As shown in all left panels, an increase in MAP was observed at all the points stimulated, with the largest response obtained at coordinate 2.1, representing the center of the NA and the C1 region ventral to NA. The right panels of Figure 4 show that at coordinate 2.1 bradycardia was found at all dorsoventral coordinates. The bradycardic response in general was small or absent at coordinate 2.0, while at the lateral coordinate 2.2, small increases in HR were observed. A similar pattern of pressor responses was obtained.
Figure 4. Hemodynamic effects of electrical stimulation of the nucleus ambiguus and the C1 area in three anteroposterior (AP) sections: coronal sections of the medulla 3.6 (A), 3.8 (B), and 4.0 mm (C) posterior to the interaural line. Each part illustrates the 15 sites stimulated in the nucleus ambiguus and the C1 area. Left panels show the MAP responses of each of 15 sites stimulated in three lateral (2.0, 2.1, 2.2) and five dorsoventral (+0.4, +0.2, 0.0, −0.2, −0.4) coordinates in each anteroposterior section. Right panels show HR responses of each of 15 sites stimulated at the same coordinates. The results are the means ± SEM of eight rats weighing 337 ± 6 g.
when coordinates $-3.6$ and $-4.0$ were stimulated; however, the bradycardic responses were much smaller or virtually absent (see Figure 4A and C).

**Discussion**

The part of the NA that we stimulated or lesioned (1300 to 1700 $\mu$m rostral to the obex) corresponds to the ventrolateral responsive locus described by Nosaka et al., which extends from the level of the obex to 2000 $\mu$m rostral from obex. The area studied also corresponds to that investigated by Stuesse and Fish, who showed that cardioinhibitory cells in the ventral medulla were found in the rostral NA. In a recent study, Bieger and Hopkins showed that, although the rostral NA is rich in neurons projecting to the larynx, this region also includes the external formation of the NA, a ventral division of the NA that extends along the entire length of the medulla and contains preganglionic neurons innervating the heart.

The lesion of cell bodies of the NA with KA reproduced our earlier results obtained with electrolytic lesion. These findings show that the enhanced increase of AP in SAD rats with previous lesion of the NA is related to lesion of cell bodies and not to fibers of passage. Rats studied 3 days after injection of KA showed no changes in MAP and HR, indicating that this region probably does not exert a tonic inhibitory influence on the sympathoexcitatory systems. The KA and electrolytic lesion did, however, produce functional changes that included a reduction in intrinsic HR and a significant reduction in the reflex bradycardic response to an increase of AP produced by phenylephrine.

Previous studies showed that KA administered into the nucleus tractus solitarii produced blockade of the baroreceptor reflex and neurogenic hypertension. In other studies from our laboratory we observed that KA injected into the NA produced within 1 hour a slight increase in AP and HR (unpublished observation, 1987), suggesting a direct initial excitatory effect on sympathetic neurons localized in that area or, alternatively, removal of an inhibitory influence of the NA on other sympathoexcitatory neurons.

Glutamate has been suggested as the neurotransmitter released by baroreceptor afferent nerves, and the presence of glutamate receptors in the medullary ventrolateral vasopressor and vasodepressor areas has also been described. In 1987, Guynet et al. suggested that a glutamatergic excitatory transmitter is involved in the VLM in mediating or modulating vagal and sympathetic reflexes. Our results with injection of glutamate into the NA suggest that this area contains two types of neurons: one type related to sympathetic excitation (pressor response) and the other to cardioinhibitory neurons (bradycardia). Since bradycardia was not observed with the smaller dose of glutamate the cardioinhibitory neurons may be less sensitive to chemical excitation than are the pressor neurons. An alternative explanation is that the higher concentration of glutamate diffused to another site; however, the different response obtained with injection of glutamate into the closely situated C1 region suggests that this may not be the mechanism.

The NA has been also suggested as the site of a GABA receptor–mediated inhibition of vagal tone. Although the site of action was not determined, Antonacci and Taylor suggested that bradycardia induced by a GABA agonist injected in the cerebroventricular route is caused by inhibition of sympathetic outflow. Our results with GABA injected into the NA and the C1 area were similar in relation to bradycardia and hypotension. On the basis of the experiments showing that bradycardia produced by GABA administered in the NA area was abolished in rats pretreated with propranolol, we suggest that the GABA effect in the NA is produced by inhibition of sympathetic outflow.

When glutamate was injected into the C1 area, the increases in MAP and HR in response to both doses were similar. These results, when compared with the NA injection, indicate that the bradycardia observed with glutamate at a dose of 50 nmol/100 nl was related to receptors located in the NA because tachycardia was observed when the C1 area was stimulated with the same dose. From these qualitatively different HR responses to microinjections centered 800 $\mu$m apart, we can assume that the spread of glutamate probably did not exceed approximately one-half that distance. The HR responses to glutamate and GABA injection into the NA and the C1 area in SAD rats were attenuated when compared with those of normal rats, suggesting a possible change in central mechanisms of HR control in neurogenic hypertension.

The mapping of the NA and the C1 area showed that all of the 45 points stimulated produced a pressor response, with the greatest effects observed when the points centered in the NA and the C1 area (coordinate 2.1) were stimulated. The bradycardic response was also most prominent at this coordinate. We believe that the bradycardia observed when coordinates below the NA (i.e., 0.0, −0.2, and −0.4 mm dorsoventral) were stimulated probably resulted from stimulation of fibers of passage from the NA. This hypothesis is supported by the results obtained with chemical stimulation of the cell bodies of this area with glutamate which failed to induce bradycardia. We believe that current spread probably does not account for the differences in cardiovascular responses seen between the various points that were mapped. Stimulation of lateral coordinate 2.2 produced an increase of MAP and tachycardia. The results of stimulation of rostral section −3.6 and caudal −4.0 showed pressor responses at all points stimulated; however, the bradycardic responses in both cases were significantly smaller than those seen at coordinate −3.8, suggesting that the HR response is restricted to a thin column of neurons from the NA to the C1 area.

The results of mapping this area with microelectrode stimulation and microinjection of glutamate and GABA show that the NA is associated anatomically with pressor sites whose neuroanatomical connections, for example, with VLM, need to be established. The
findings with KA-induced lesion of the NA suggest that removal of cell bodies in this area can alter an inhibitory influence on sympathetic vasomotor tone. The loss of this inhibition could provide a mechanism for the enhanced increase of AP seen after baroreceptor deafferentation. We conclude that the NA is involved not only in HR control but also in central regulation of AP through pressor systems, and perhaps separately with neuronal projections associated with the baroreceptor reflex.

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