Mechanisms of Cardiovascular Responses to Glycine Injected Into the Dorsal Vagal Motor Nucleus in Rat

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Microinjection of glycine into the dorsal vagal motor nucleus of anesthetized rat elicits increases in arterial pressure and heart rate. In the nucleus tractus solitarii, where cardiovascular responses to injection of glycine may be mediated through release of acetylcholine, there is a dense concentration of glycinergic nerve terminals and glycine receptors. In this study, using immunohistochemical methods, we show that glycine terminals and receptors are present in caudal dorsal vagal motor nucleus, although the concentration of both terminal elements is less than in adjacent nucleus tractus solitarii. Responses to glycine microinjected into the dorsal vagal motor nucleus are blocked by the muscarinic antagonist atropine microinjected at the same site; but, unlike responses to glycine in the nucleus tractus solitarii, responses to glycine in the dorsal vagal motor nucleus are not prolonged by physostigmine. These data support the possibility that endogenous glycine may play a role as a transmitter in the dorsal vagal motor nucleus. Responses to glycine may be mediated through actions at muscarinic receptors but not through acetylcholine itself.

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We have previously shown that microinjection of glycine into the dorsal vagal complex, composed of the nucleus tractus solitarii (NTS) and dorsal vagal motor nucleus (DMV), elicits site-specific changes in arterial pressure and heart rate. In NTS, glycine decreases both arterial pressure and heart rate, whereas injections into the region of the DMV raise both pressure and heart rate. We have shown that there is a dense concentration of glycine immunoreactive receptors and terminals in NTS and that responses to glycine in NTS are mediated through cholinergic receptors and possibly through release of acetylcholine. In the present study, we have sought to determine if similar glycine synaptic elements exist in DMV and if responses to exogenously administered glycine are mediated through cholinergic mechanisms.

Methods

Pharmacological Methods

Eight adult male Sprague-Dawley rats (Bio-Lab Corp., St. Paul, Minn.) were anesthetized with halothane (1.5–2.0%) in 100% oxygen administered by a nose cone. Arterial pressure was recorded via a cannula passed into a femoral artery and connected through a strain-gauge transducer (Statham P23Db, Statham Division, Gould Inc., Oxnard, Calif.) to a polygraph. Heart rate measurements were derived from the arterial pulse wave by a cardiotachometer.

The dorsal surface of the medulla was exposed through a dorsal midcervical incision and a partial occipital craniotomy. Glass multibarrel pipettes (approximately 20 μm o.d.) were filled with solutions containing glycine (400 mM), physostigmine (0.6 mM), or atropine (1.5 mM) and vehicle (artificial cerebrospinal fluid containing concentrated methylène blue). The artificial cerebrospinal fluid was composed of (mM) NaCl 132, KCl 3, CaCl2 1.5, MgCl2 0.65, NaHCO3 25, and dextrose 3.7, pH 7.3. The concentration of glycine was the concentration that we had previously found to elicit maximal responses on injection into DMV. The concentrations of physostigmine and atropine were those that maximally and specifically affected glycine and acetylcholine when those agents were injected into NTS.

The filled micropipettes were stereotactically placed into DMV, and a 25-nl volume of solution was injected over 1–2 seconds by a WPI picopump (New
Haven, Conn.). Volumes of injectate were confirmed by visualizing the movement of the meniscus in the pipette through a microscope equipped with a reticulated ocular. Reproducibility of glycine responses was confirmed with at least two injections before the effects of atropine or physostigmine on glycine-mediated responses were studied. Responses to glycine were again tested immediately after the injection of either physostigmine or atropine. As we found no effect of prior injection of artificial cerebrospinal fluid on glycine responses, data from those control experiments are not reported. At the end of each study, 20 nl methylene blue solution was injected to confirm histologically at postmortem examination that injections lay in the DMV. Thus, injection sites were confirmed both histologically and by the physiological responses seen. At the end of all experiments, animals were killed while still anesthetized by intravenous injection of KCl.

Data from these studies are expressed as mean±SEM and were analyzed by dependent t test for paired functions.

**Immunohistochemical Methods**

In separate studies, glycine and glycine receptor immunoreactivity was examined in the DMV. Anesthetized rats (n=9) were killed by exsanguination, and tissue was fixed by the intracardiac perfusion of 3% glutaraldehyde in phosphate buffered saline. The brains were removed and 10- and 50-μm sections through the medulla were cut on a Vibratome for light and electron microscopic evaluation, respectively. Sections were incubated for 24 hours either in a rabbit antiserum to glycine (dilution, 1:1,000; supplied by R. Wenthold, National Institutes of Health) or in a rat antiserum to glycine receptors (dilution, 1:1,000; Boehringer Mannheim, Indianapolis, Ind.). Immunoreactivity was detected using peroxidase-antiperoxidase immunohistochemistry with 3,3'-diaminobenzidine as the chromagen. Selected 50-μm sections were subsequently treated with osmium, flat-embedded in Spurr's medium, and processed for ultrastructural examination. Before ultrathin sectioning, blocks were beveled to preserve tissue orientation. Semithin sections (0.5 μm) were also taken, stained with toluidine blue, and used to locate the DMV.

All methods used in these studies were approved by the Committee on Animal Use of The University of Iowa, and the Veterans Affairs Research and Development Service.

**Results**

We first sought to determine if glycinergic terminals and glycine receptors could be found in the DMV to account for the observed cardiovascular responses to glycine and to suggest a role for endogenous glycine in cardiovascular control at the level of the DMV. Using an antibody to glycine, we found a low concentration of glycine immunoreactivity in DMV, particularly compared with that found in the adjacent NTS and hypoglossal nucleus (Figure 1). The immunoreactivity was densest in caudal DMV and sparse in the rostral two thirds of DMV. As we have previously shown in the NTS, glycine immunoreactivity in DMV was found largely in terminals containing pleomorphic vesicles (Figures 2A and 2B). Occasionally, immunoreactive terminals containing dense spherical vesicles were observed. Almost all glycine immunoreactive terminals in DMV appeared to be in contact with dendrites. In several instances, glycine immunoreactive terminals were observed in contact with immunoreactive dendrites (Figure 2B).

At similar levels of the brain stem, glycine receptor immunoreactivity was densely concentrated in DMV, as it was also in the NTS and hypoglossal nucleus (Figure 2C). Ultrastructurally, glycine receptor immunoreactivity was characterized by an electron dense patch subjacent to the plasma membrane of dendrites (Figure 2D). In every case, this staining was located adjacent to terminals that contained pleomorphic vesicles similar to those shown to contain glycine immunoreactivity (asterisks in Figure 2D).

To determine if responses to injection of glycine (400 mM) into DMV, like those we have demonstrated when glycine is injected into NTS, were mediated through cholinergic mechanisms, we next studied the effects of atropine on responses to glycine.

In five rats, microinjection of atropine (37 pmol; 1.5 mM) from one barrel of a multibarrel pipette transiently eliminated the pressor response to glycine injected from an adjacent pipette (Figure 3). Responses returned within 15 minutes. Often after atropine, glycine elicited decreases in arterial pressure and heart rate that were qualitatively like those seen when glycine was injected into NTS. Thus, the response to glycine before atropine was an increase of arterial pressure and heart rate of 36±8 mm Hg and 44±7 beats per minute, respectively; after atropine, it was a decrease of 13±4 mm Hg and 13±4 beats per minute, respectively (p<0.05).

If the effects of glycine were due to release of acetylcholine, as may be the case in NTS, then those effects should be prolonged if metabolism of acetylcholine were blocked. In contrast to NTS, where the effects of glycine are prolonged by microinjection of physostigmine before glycine, the cholinesterase inhibitor (16 pmol) injected into DMV of four animals before glycine (10 nmol) did not affect the duration of responses (total time of elevation of arterial pressure and heart rate above baseline) to glycine (before physostigmine, 10±2 seconds; after, 10±1 seconds). The amplitude of responses was also unchanged by physostigmine.

**Discussion**

This study confirms the presence of glycine immunoreactive terminals and receptors in the region of DMV where pressor/tachycardiac responses can be elicited by the microinjection of glycine. Glycine terminal labeling in the DMV has not previously been identified. The present data suggest that a...
graded level of terminal density is present. In the rostral two thirds of DMV, glycine terminals are very sparse, contrasting with heavy labeling in NTS and hypoglossal nucleus.\(^3\) Caudally, however, significant numbers of terminals are identified, although the density of labeling is less than that seen in the NTS. This graded density may reflect preferential contact with a specific subpopulation of DMV neurons. Previous studies\(^8\) have suggested that cardiomotor neurons are located in caudal parts of DMV. However, the low levels of glycine terminals at rostral regions does not preclude contacts between DMV neurons.
and glycine terminals. The DMV is predominantly composed of cell bodies of large preganglionic neurons. As our ultrastructural studies indicate that glycine terminals are largely associated with dendrites, glycine terminals may contact DMV neuronal dendrites in adjacent regions of NTS and reticular formation.

Autoradiographic studies using tritiated strychnine have suggested the presence of glycine receptors in DMV. The present finding of high levels of glycine receptor immunoreactivity in DMV is consistent with that suggestion. Whether the antibody recognizes a strychnine-sensitive or -insensitive receptor remains to be determined. However, it is most probable that strychnine-sensitive receptors are being recognized, because all of the cardiovascular responses to glycine injected into DMV are blocked by that antagonist.

The present data provide indirect evidence that glycine terminals on dendrites in DMV make synaptic contact with postsynaptic regions, demonstrating glycine receptor immunoreactivity. Although a double labeling study was not performed, vesicles within terminals apposed to glycine receptor labeling are pleomorphic, as are the bulk of the glycine immunoreactive terminals we identified using the glycine antibody. Although the density of glycine terminals in DMV was considerably less than we have reported in adjacent NTS, the receptor densities were comparable. The reason for this discrepancy is unknown and may relate to the specificity of the glycine receptor antibody. There is some evidence (H.K. Kultas-Illinsky, personal communication, 1991) that, in the thalamus, immunoreactivity associated with the antibody used here is related to the N-methyl-D-aspartic
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Glycine and Dorsal Vagal Motor Nucleus

Arterial Pressure (mmHg)
Mean Arterial Pressure (mmHg)
Heart Rate (bpm)

BEFORE ATROPINE

AFTER ATROPINE

GLY

GLY

1 min

FIGURE 3. Representative tracings show blockade by atropine of the reproducible pressor and tachycardiac responses elicited by microinjection of glycine (GLY) into the dorsal vagal motor nucleus. Repeated injections of glycine (10 nmol) at 5-minute intervals unilaterally into dorsal vagal motor nucleus increased arterial pressure and heart rate, but the response was almost completely eliminated when the injection of glycine followed injection of atropine (37 pmol) at the same site. bpm, Beats per minute.

acid (NMDA) receptor, which, although capable of binding glycine, is also associated with other endogenous ligands. It is nonetheless unlikely that a binding site in the NMDA complex is being recognized, because glycine binding to that complex is not strychnine sensitive.

As we have not yet performed double labeling studies to identify both glycine immunoreactivity and choline acetyltransferase immunoreactivity in the same section, we do not know the relation between glycine terminals and cholinergic neuronal elements in DMV. It is well recognized that preganglionic vagal neurons in DMV stain positively for choline acetyltransferase; thus, synaptic contacts between glycergic and cholinergic elements may be found in the DMV or in the NTS. However, this study provides several lines of evidence suggesting that, in DMV, glycine does not mediate its response through modulation of release of acetylcholine. First, acetylcholine itself did not consistently produce a pressor response, as did glycine. Second, the duration of responses to glycine was not affected by phystostigmine, which would block metabolism of acetylcholine. Release of acetylcholine at the synaptic cleft on exposure of cholinergic neurons to glycine would still be possible if the synaptic terminal in DMV were protected from the cholinesterase inhibitor or the postsynaptic membrane were not as accessible to exogenous, as it is to endogenous, acetylcholine. The latter seems an unlikely explanation for the lack of response to acetylcholine, because acetylcholine injected into NTS produces a consistent response. It is possible that the response to glycine, which was shown here to be blocked by muscarinic blockade, may be effected through direct action of glycine on the muscarinic receptor complex. Such a modulatory role has been shown for glycine at the NMDA receptor and may, likewise, occur at the muscarinic receptor.

Although atropine can produce local anesthesia, it is doubtful that this was the case in these studies. The dose of atropine was the same as used in previous experiments in NTS in which it selectively blocked acetylcholine and glycine but did not affect the response to glutamate and did not block the baroreceptor reflex.

In conclusion, the present study demonstrates glycergic terminals and receptors in DMV and thus supports a role for endogenous glycine as a transmitter in that nucleus. Although glycine may mediate cardiovascular responses on injection in DMV by an action on muscarinic receptors, it does not seem that those responses depend on acetylcholine release, as may be the case in NTS.

Acknowledgments

We gratefully acknowledge the secretarial support provided by Jean Hulme and the technical support provided by Lisa Roberts in the preparation of the manuscript.

References


**KEY WORDS** • glycine • acetylcholine • immunocytochemistry • vagus nerve • blood pressure • heart rate
Mechanisms of cardiovascular responses to glycine injected into the dorsal vagal motor nucleus in rat.
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Hypertension. 1992;19:II187
doi: 10.1161/01.HYP.19.2_Suppl.II187

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