Glycine Microinjected in the Rat Dorsal Vagal Nucleus Increases Arterial Pressure

WILLIAM T. TALMAN

SUMMARY Microinjections (25 nl) of glycine into the dorsal motor nucleus of the vagus in 21 rats elicited dose-dependent increases of arterial pressure and heart rate that were not seen with injections adjacent to the dorsal motor nucleus of the vagus. The responses to glycine were neurally mediated and could be blocked either by local pretreatment with strychnine or by combined vagotomy and ganglionic blockade. The data suggest that glycine receptors on, or in the region of, neurons of the dorsal motor nucleus of the vagus may have a role in the regulation of arterial pressure and heart rate. (Hypertension 11: 664–667, 1988)

KEY WORDS inhibitory amino acids • hypertension • baroreceptor • tachycardia • strychnine • nucleus tractus solitarii

The role in cardiovascular regulation played by glycinergic mechanisms in the dorsal vagal complex has received little attention. We have previously shown1 that glycine microinjected into the cardiovascular portion of the nucleus tractus solitarii (NTS) elicited hypotension and bradycardia. Microinjections ventral to that nucleus produced pressor responses and tachycardia. Others2 have demonstrated pressor and tachycardiac responses when glycine was injected into the dorsal vagal complex. In this study we sought to determine the cardiovascular effects of the microinjection of glycine into the dorsal motor nucleus of the vagus (DMV) and ventral-most portions of the NTS.

Materials and Methods

Twenty-one adult male Sprague-Dawley rats (Biolabs, St. Paul, MN, USA) were anesthetized with halothane (1.5%) delivered in 100% oxygen through a nasal mask. The animals were instrumented with femoral venous and arterial cannulas for intravenous delivery of drugs and for recording of intra-arterial pressure, respectively. Arterial pressure (AP), mean arterial pressure (MAP), and heart rate (HR), calculated by a tachygraph from the arterial pulse wave, were displayed on channels of a polygraph (Model 7, Grass, Quincy, MA, USA). Animals were placed in a stereotaxic frame (David Kopf, Tujunga, CA, USA), and the dorsal surface of the medulla was exposed at the level of the calamus scriptorius. A glass micropipette (tip outside diameter, 40–50 μm) was lowered into the brainstem at predetermined coordinates. The pipette was connected by plastic tubing either to a 1-μl syringe (Pressure Lok, Dynatech, Baton Rouge, LA, USA) or to a pneumatic ejection system (Pneumatic Picopump, WPI, New Haven, CT, USA) that was used to eject 25-nl volumes of fluid. The injected volume was monitored through a microscope with a reticle.

Microinjunctes consisted either of vehicle (artificial cerebrospinal fluid) or solutions of glycine, glutamate, or strychnine. Multibarrel pipettes were used to eject different agents at the same site. For the development of dose-response curves, glycine was injected from a single-barrel pipette that was removed from the brainstem after each dose, washed with artificial cerebrospinal fluid, filled with the next highest concentration, and placed at the same location through the visually identified pipette track for delivering the next dose. The final dose of each agent was delivered in a concentrated solution of methylene blue to mark the injection site.

Statistical analyses were performed using Student’s t test for paired and unpaired functions as indicated. All data were expressed as means ± SEM.

Results

Microinjections of glycine, but not vehicle, into the DMV region immediately increased both AP and HR (Figure 1). Occasionally, a transient late (onset, 30 seconds to 1 minute after injection) pressor response...
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Control Glycine Glycine

Pulsatile
Arterial
Pressure
(mmHg)

Mean
Arterial
Pressure
(mmHg)

Heart
Rate
(bpm)

FIGURE 1. Glycine (8 nmol) but not vehicle (control), microinjected (inj) into the dorsal motor nucleus of the vagus, increased arterial pressure and heart rate in two rats.

followed an intermediate depressor response. The consistent early pressor effect was used to generate dose responses (Figure 2). The threshold dose for the initiation of pressor effects and increases of HR was 1 nmol. The maximally effective dose of 8 nmol elicited a pressor response of 31.2 ± 4.8 mm Hg from a baseline MAP of 80.8 ± 6.2 mm Hg and a tachycardic response of 30.6 ± 5.7 beats/min from a baseline HR of 300.0 ± 39.3 beats/min (n = 9). The response to a dose was reproducible and was the same whether given as the first or the last of a series of different doses. In contrast to the effects of glycine, the injection of L-glutamate (200 pmol) at the same site produced either no response or a small (5-10 mm Hg and 5-10 beats/min) decrease of AP and HR.

The cardiovascular response to the injection of supramaximal (15 nmol) doses of glycine was abolished by strychnine (67 pmol) injected at the same site immediately before glycine (Figure 3). The blockade was transient; responses to glycine returned within 10 minutes of the injection of strychnine.

The effects mediated by the microinjection of glycine were not attenuated by bilateral cervical vagotomy, but when propranolol (1 mg/kg i.v.) was administered to vagotomized animals, the tachycardic response to glycine was completely abolished (Table 1). Combined vagotomy and ganglionic blockade (chlorisondamine, 2.5 mg/kg i.v.) abolished both the AP and HR responses (Table 2).

Postmortem histological examination confirmed that injection sites defined by the diffusion sphere (approximately 0.2 mm) of methylene blue, confined to the DMV-ventral NTS, produced pressor and tachycardic responses, while injections administered intravenously into the cisterna magna or into the dorsal half of the brainstem outside that region did not (Figure 4). As previously reported,1 injections confined to the NTS, as little as 0.2 mm dorsal to the DMV, produced depressor and bradycardic responses in contrast to the responses seen with injections into the DMV. Occasionally, injections into the ventral NTS-DMV region elicited both pressor and depressor responses.

Discussion

The inhibitory amino acid glycine has important cardiovascular modulatory effects in the ventral medulla,3-5 but its role in circulatory control by the dorsal vagal complex has received little attention. Some studies have suggested a possible glycinergic mechanism in cardiovascular control by the dorsal medulla. For example, glycine has been found in high concentrations in the dorsal vagal complex,2-6 and its release from a neurotransmitter pool within the dorsal vagal complex2-6 and inactivation by synaptosomal high affinity uptake8 have been demonstrated. Previously, we have shown1 that the microinjection of glycine into the cardiovascular portion of the NTS depresses both AP and HR. Others have demonstrated an increase of AP and HR.3 The current studies show that the response to glycine is highly site-specific and that microinjections of the amino acid confined to the DMV and ventralmost portions of the NTS increase AP and HR. The
Table 1. Vagal and β-Adrenergic Influences on Cardiovascular Responses to Glycine (10 nmol) in the Dorsal Motor Nucleus of the Vagus of Five Rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before vagotomy</th>
<th>Before β-blockade</th>
<th>After β-blockade</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔMAP (mm/Hg)</td>
<td>+27.0 ± 4.6 (81.2 ± 6.6)</td>
<td>+31.8 ± 4.0 (80.6 ± 5.6)</td>
<td>+23.4 ± 4.3* (77.0 ± 10.6)</td>
</tr>
<tr>
<td>ΔHR (beats/min)</td>
<td>+27.0 ± 9.7 (335.8 ± 41.8)</td>
<td>+30.0 ± 12.2 (375.6 ± 31.5)</td>
<td>+5.0 ± 2.7* (325.6 ± 62.6)</td>
</tr>
</tbody>
</table>

Values are means ± SEM from studies of five rats. Figures in parentheses are baseline values. *p < 0.05, compared with values before β-blockade.

Table 2. Vagal and Sympathetic Influences on Cardiovascular Responses to Glycine (10 nmol) in the Dorsal Motor Nucleus of the Vagus of Five Rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before vagotomy and chlorisondamine</th>
<th>After vagotomy and chlorisondamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔMAP (mm/Hg)</td>
<td>+23.8 ± 4.3 (80.8 ± 1.5)</td>
<td>-2.9 ± 1.1* (75.0 ± 2.0)</td>
</tr>
<tr>
<td>ΔHR (beats/min)</td>
<td>+38.8 ± 10.9 (333.8 ± 2.4)</td>
<td>-1.4 ± 1.5* (322.5 ± 11.1)</td>
</tr>
</tbody>
</table>

Values are means ± SEM from studies of five rats. Figures in parentheses are baseline values. Arterial pressure was maintained at control levels by phenylephrine, 6.3 μg/kg/min i.v., after vagotomy and chlorisondamine. *p < 0.01, compared with prevagotomy values.

Figure 4. Diagrammatic representations of transverse sections 0.2 mm rostral (top) and caudal (bottom) to the calamus scriptorius showing sites responsive to 10 nmol of glycine and the changes in arterial pressure and heart rate that were elicited. Injections administered intravenously into the cisterna magna or into sites outside the dorsal vagal complex did not change arterial pressure or heart rate. In contrast, injections into the dorsal motor nucleus of the vagus (X) produced hypertension and tachycardia, dorsal NTS injections produced hypotension and bradycardia, and injections into both areas gave mixed responses. The shaded areas depict the diffusion spheres of methylene blue at each representative site. AP = area postrema; C = central canal; TS = tractus solitarius; XII = hypoglossal nucleus.

precise localization of the neurons responsible for these cardiovascular responses cannot be determined with any greater precision from these studies. Therefore, we cannot know whether the responses are solely mediated through actions on the cell somata within the DMV or whether, instead, dendrites or cells within the ventral-most portion of the NTS are responsible. The occurrence of pressor responses followed by depressor responses when injections involved both the DMV and cardiovascular NTS suggest to us that at least two sites in the vagal complex may be affected by the glycine injections, one in or near the DMV and one more dorsally in the NTS. The previously reported dominant pressor responses to glycine injections in the NTS may have actually resulted from injections in the NTS-DMV region, since the injection sites were marked by an electrolytic lesion made through an electrode placed at the same coordinate, but perhaps not the same site, as the injection. In our studies the vital stain was injected at the site from which the physiological response was elicited.

Clearly, the pressor and tachycardic responses to glycine are neurally (and apparently sympathetically) mediated. Both are eliminated by vagotomy and ganglionic blockade, while vagotomy alone has no effect. It is unclear through what central neural pathways these sympathetically mediated responses are generated. There is no evidence for direct projections from the DMV to the intermediolateral column in the spinal cord,² the site of sympathetic preganglionic neurons, although sparse projections to the thoracic spinal cord have been demonstrated.¹⁰ Potentially, projections from the DMV through the carotid sinus nerve¹¹ or through the aortic depressor nerve (not yet described) could produce the cardiovascular effects through some effect on the baroreceptors. More likely, the physiological responses could result from influences of the pressor region on relay nuclei such as the caudal ventrolateral medulla or the rostral ventrolateral
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medulla. The latter may be a tonic vasomotor center, while the former is a depressor zone that may effect cardiovascular changes through both sympathetic and humoral mechanisms. Both the caudal ventrolateral medulla and the rostral ventrolateral medulla receive projections from the dorsal vagal complex. However, since the bulk of the neurons projecting from the dorsal vagal complex to the caudal ventrolateral medulla lie more dorsally and in an area homologous to that from which hypotensive and bradycardic responses can be elicited, the rostral ventrolateral medulla projections seem more likely to be involved.

We cannot determine from the current data the physiological mechanisms mediating the responses to glycine. Glycine is generally considered a major inhibitory neurotransmitter in the central nervous system, although excitatory responses have been described. The contrasting effects of glycine and glutamate in this glycine pressor region of the dorsal vagal complex does suggest that glycine may have inhibited local neurons.

Despite the limitations on interpretation of these data at a mechanistic level, the study does demonstrate several important observations. First, glionicergic mechanisms, through a presumed activation of glycine receptors in the dorsal vagal complex, may be involved in central cardiovascular control. Second, an area in or immediately adjacent to the DMV responds to glycine and causes sympathetically mediated hypertension and tachycardia.

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