Hemodynamic Effects Elicited by Stimulation of the Nucleus Tractus Solitarii

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Abstract Microinjection of the excitatory amino acid L-glutamate into the nucleus tractus solitarii (NTS) elicits decreases in arterial pressure and heart rate. In the present study, we sought to determine the regional hemodynamic effects that were correlated with changes in arterial pressure and heart rate produced by stimulation of the NTS. In anesthetized rats, blood flow in the renal (RBF), superior mesenteric (MBF), and hindquarter (HBF) vascular beds was measured by pulsed Doppler flowmeters. Relative vascular resistances (RVR, MVR, and HVR) were calculated by dividing mean arterial pressure (mm Hg) by the Doppler shift (kHz). Microinjection of L-glutamate into the NTS caused rapid, transient, dose-related decreases in mean arterial pressure and heart rate. MVR and RVR were minimally changed immediately after injections, but both demonstrated delayed dilatation. In contrast, HVR fell immediately but demonstrated delayed constriction. Identical changes occurred in intact rats and in those with interruption of the baroreflex by sinoaortic denervation. Ganglionic blockade with hexamethonium abolished virtually all L-glutamate-induced responses. This study suggests that NTS neurons exert differential effects on renal, mesenteric, and hindquarter vascular beds and that glutamate-induced regional hemodynamic changes are mediated predominantly through autonomic pathways. (Hypertension. 1994;23[suppl I]:I-73-I-77.)

Key Words • glutamate • blood flow • blood pressure • hemodynamics

Glutamate (GLU) microinjected into the cardiovascular region of the nucleus tractus solitarii (NTS) of anesthetized rats elicits decreases in arterial pressure (AP) and heart rate (HR). These initial physiological observations together with pharmacologic, anatomic, and biochemical data have strongly supported the hypothesis that GLU is an important neurotransmitter in the NTS. It is generally presumed that the depressor responses elicited by GLU result from uniform peripheral vasodilatation due to withdrawal of sympathetic drive and that bradycardia results from increased cardiac parasympathetic activity. However, hemodynamic mechanisms associated with GLU stimulation of the NTS have not been studied. Most studies of the role played by the NTS in regulating cardiovascular function have analyzed only AP and HR, and occasionally sympathetic activity. Recent studies have suggested that stimulation of central sites may effect hemodynamic changes that differ from one vascular bed to another. The magnitude and time course of changes in blood pressure induced by stimulation of different brain sites may relate to different profiles of regional hemodynamic response. Central regions may provide such distinctive modulation of end-organ function through selective innervation of specific clusters of preganglionic neurons in the spinal cord. Therefore, we hypothesize that excitation of neurons in the NTS by microinjection of GLU may lead to a complex pattern of hemodynamic changes rather than a global reduction in peripheral resistance in all vascular beds. Thus, we have sought to analyze peripheral hemodynamic effects of excitation of NTS neurons by microinjection of GLU into anesthetized rats.

Methods

Twenty-one adult male Sprague-Dawley rats were anesthetized with halothane (1.5%) delivered in 100% oxygen. In five rats, the baroreflex was interrupted by sinoaortic denervation effected by bilateral transection of the aortic depressor, carotid sinus, and superior laryngeal nerves. Animals were instrumented with a femoral venous cannula for administration of drugs and with a femoral arterial cannula connected via a pressure transducer (Statham P23ID, Statham Division, Gould Inc, Oxnard, Calif) to a low-level direct-current preamplifier for recording AP and mean AP (MAP) on a polygraph (model 7D, Grass Instrument Co, Quincy, Mass). HR, calculated with a tachygraph triggered by the arterial pulse wave, was continuously recorded. A midline laparotomy was performed to expose the left renal artery, superior mesenteric artery, and lower abdominal aorta. A miniaturized pulsed Doppler flow probe was placed around each artery. Signals (kHz) from pulsed Doppler flow probes correlate with blood flow. Probes were connected to a directional pulsed Doppler flowmeter (model 545C-4, Bioengineering, The University of Iowa, Iowa City, Iowa) to allow continuous recording of renal, superior mesenteric, and hindquarter blood flows (RBF, MBF, and HBF, respectively) on the polygraph. Relative vascular resistances (RVR, MVR, and HVR) were calculated (in mm Hg/kHz). Before experiments, each channel of the polygraph was calibrated and pens were precisely synchronized.

Approximately one half hour after completion of instrumentation, adequacy of the preparation was confirmed by recording all variables at a fast paper speed that allowed analysis of phasic blood flow signals with regard to blood pressure (Fig 1, left panel). In all subsequent protocols, data were analyzed...
from averaged signals produced by setting a 0.5-Hz frequency response on the amplifier of the polygraph (Fig 1, right panel).

Animals were placed in a stereotaxic frame, and the dorsal surface of the medulla oblongata was exposed at the level of the calamus scriptorius through a partial occipital craniotomy. At predetermined coordinates in the NTS, microinjections were made through glass micropipettes connected to a pneumatic pressure system (model PPS-2, Medical Systems Corporation, Greenvale, NY). Dose-related responses to GLU were determined by varying concentrations of GLU in a fixed volume (50 nL) of injectate. GLU was dissolved in artificial cerebrospinal fluid that was used alone as a sham control injection. Injection sites were marked for subsequent histological localization with methylene blue dissolved in artificial cerebrospinal fluid and injected through an adjacent barrel of the multibarrel pipette. All data were expressed as mean±SEM of percent change, and data were analyzed with Student’s t test for paired functions. Flow data were expressed as shift of Doppler signals (kHz).

Results

In nine intact rats, hemodynamic responses to three doses of GLU (50, 100, and 200 pmol) were determined. Microinjection of GLU into the NTS elicited previously described dose-dependent depressor and bradycardiac responses and concomitant changes in each vascular bed. Because hemodynamic changes differed between vascular beds, data were analyzed at several times during the course of responses (Figs 1 and 2). The first phase of data acquisition after injection of GLU recognized the period at which MBF had decreased to its lowest level and HBF to its highest level with regard to baseline. Both responses occurred concomitantly before MAP fell to the lowest level. The second phase of data analysis came when MAP reached its nadir. The third phase covered the period when MBF reached its highest level and HBF reached its lowest level. During each phase of response, as well as the baseline period and the period of recovery after responses, each variable was derived from the same time point.

Each dose of GLU elicited biphasic changes of MBF and HBF (Fig 2). After injection of 100 pmol of GLU, MBF decreased (from 3.7±0.2 to 2.8±0.2 kHz) during phase 1 and increased (from 2.8±0.2 to 4.3±0.3 kHz) during phase 3 of data acquisition. The initial decrease in MBF was associated with a slight increase in MVR and occurred before the maximal fall in MAP. The subsequent increase in MBF was associated with reduced MVR and occurred as MAP returned approximately halfway to baseline. Decreases in MBF and MVR were linearly related to dose, but the increases in MBF and MVR were inversely related to dose.

HBF and HVR responses were also biphasic; however, after a 100-pmol dose of GLU, HBF increased (from 3.9±0.2 to 4.0±0.4 kHz) and HVR decreased (from 25.4±3.3 to 18.7±3.1 mm Hg/kHz) during the first phase of data acquisition. In contrast, HBF decreased (from 4.0±0.4 to 2.9±0.4 kHz) and HVR increased (from 18.7±3.1 to 27.9±5.2 mm Hg/kHz) during the third phase. Increases in HBF and HVR
were inversely related to dose, whereas the decreases of both variables were directly related. The maximal fall in HVR followed injection of 200 pmol of GLU, whereas HVR increased maximally in phase 3 after a 50-pmol dose of GLU. Unlike other vascular beds, changes in RBF were monophasic. RBF showed dose-dependent decreases after injection of GLU. Calculated RVR did not change during initiation of depressor responses; however, during recovery of depressor responses, RVR was reduced in a dose-dependent manner.

Responses were also studied in five animals after complete transection of aortic depressor, superior laryngeal, and carotid sinus nerves. The pattern and temporal sequence of responses (not depicted) were identical to those seen in rats with an intact arterial baroreflex.

Ganglionic blockade with hexamethonium (10 mg/kg IV) in seven rats reduced MAP from 100±1.0 to 74±4.0 mm Hg (P<.05) and HVR from 18.0±1.7 to 14±1.6 mm Hg/kHz (P<.05); however, HR, RVR, and MVR were not changed. Hexamethonium nearly eliminated all responses of MAP, HR, and regional vascular resistances elicited by GLU (100 pmol) injection (Fig 3).

Discussion

The present study makes three contributions to our understanding of cardiovascular responses elicited by stimulation of the NTS. First, hemodynamic responses are not uniform among all vascular beds. Second, the contribution to effects on AP of changes in peripheral resistance in each vascular bed varies over time after stimulation of NTS. Finally, hemodynamic responses as well as responses of AP and HR are neurally mediated.

It has been known for more than 10 years that activation of neurons within the NTS by microinjection of GLU or its analogues elicits depressor and bradycardic responses accompanied by decreased sympathetic nerve activity, but this report provides direct evidence for regional hemodynamic effects associated with reductions in AP. An earlier study in which hemodynamic changes were studied after lesions of NTS suggested that hypertension resulting from inactivation of NTS neurons was associated with global, although variable, increases in peripheral resistance in all vascular beds. These observations suggest that depressor responses elicited by NTS stimulation result from decreased peripheral resistance in all vascular beds. It may be that...
the direction of changes in vascular resistance after NTS lesions also vary regionally but that measurements of flow at only one time miss the heterogeneity. Pulsed Doppler flowmetry was used in the present study to provide continuous, on-line assessment of blood flow in the three vascular beds studied. This method has been validated and shown to accurately indicate changes in blood flow. In each study, we carefully documented correspondence of Doppler signals with simultaneous arterial pulse waves and performed experiments only when the two signals were synchronous.

This study demonstrates that of the three vascular beds studied, early vasodilatation occurred only in hindquarter vessels. Reduced resistance in that bed could have contributed to initiation of depressor responses. Although slightly increased resistances were observed in renal and mesenteric vascular beds during initiation of depressor responses, the latter may have contributed to further reduction of AP as MVR was maximally reduced when AP reached its nadir. Hindquarter vasoconstriction appears to have contributed to return of AP toward basal values in phase 3, whereas both RVR and MVR remained at their nadir. Return of all vascular resistances to baseline also led to concomitant recovery of AP to values. Because the sequence of changes in MAP, HR, and vascular resistances was identical in animals with complete interruption of the baroreflex, it is unlikely that reflex responses to changes in AP caused the vasomotor changes that were seen.

The study does not address the contribution of vascular resistance in the skin or in other organs to changes in AP or how much changes in resistance measured in three vascular beds contributed to changes in total peripheral resistance. It is likely that changes in flow and resistance in vessels supplying skin played a role in changes in AP because maximal changes in peripheral vascular resistance occurred in skin after lesions of NTS.

Changes in vascular resistance that occurred in each of the three vascular beds studied in these protocols varied from one bed to the other and contributed to depressor responses to varying degrees at different times after injection. Autoregulation of RVR appears to have contributed little to changes seen in the renal vascular bed. Instead, the early response of RVR to depressor effects of low doses of GLU was a slight increase, not a decrease, in RVR; even when higher doses of GLU led to delayed renal vasodilatation, RBF was not maintained but demonstrated dose-related reductions proportional to the depressor responses. Possibly, anesthesia interfered with renal vascular autoregulation, but GLU, by acting directly to stimulate baroreflexes centrally, may have led to baroreflex modulation of RVR.

Regional variation in responses of vascular resistance after injection of GLU is fully consistent with previous studies that have shown similar regional heterogeneity of responses with stimulation of other central sites involved in cardiovascular regulation. It is noteworthy that two of those sites were in the ventrolateral medulla, through which NTS is known to influence autonomic activity and thus lead to cardiovascular responses to baroreflex activation. Ganglionic blockade with hexamethonium almost eliminated all hemodynamic changes produced by microinjection of GLU into the NTS. Thus, cardiovascular responses to activation of NTS neurons are largely due to modulation of autonomic neural activity.
Although the findings of the present study support neurally mediated heterogenous changes in regional vascular resistance with NTS stimulation, these conclusions would be strengthened by simultaneous recording of blood flow to the skin and of sympathetic activity in both anesthetized and conscious animals that may exhibit opposite responses.  

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