Glutamate in the nucleus of the solitary tract activates both ionotropic and metabotropic glutamate receptors

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Glutamate in the nucleus of the solitary tract activates both ionotropic and metabotropic glutamate receptors. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1858–R1866, 1998.—Glutamate is the proposed neurotransmitter of baroreceptor afferents at the level of the nucleus of the solitary tract (NTS). Blockade of ionotropic glutamate receptors with kynurenic acid blocks the arterial baroreflex but, paradoxically, does not abolish the response to exogenous glutamate. This study tested the hypothesis that exogenous glutamate in the NTS activates both ionotropic and metabotropic glutamate receptors (mGluRs). In urethan-anesthetized rats, unilateral microinjections of glutamate into the NTS decreased mean arterial pressure, heart rate, and lumbar sympathetic nerve activity. The cardiovascular response to injection of glutamate was not altered by NTS blockade of mGluRs with α-methyl-4-carboxyphenylglycine (MCPG). Blockade of ionotropic glutamate receptors with kynurenic acid attenuated the response to glutamate injection. After combined NTS injection of MCPG and kynurenic acid, the response to glutamate was blocked. These data suggest that exogenous glutamate microinjected into the NTS acts at both ionotropic glutamate receptors and mGluRs. In addition, blockade of both classes of glutamate receptors is required to block the cardiovascular response to microinjection of glutamate in the NTS.

sympathetic nerve activity; blood pressure; arterial baroreflex; rat

The arterial baroreflex is a primary mechanism by which the central nervous system regulates arterial pressure on a beat-to-beat basis. Baroreceptor afferent neurons terminate in the medial nucleus of the solitary tract (NTS), and data suggest that these afferents release glutamate as their primary neurotransmitter (12, 25). Glutamate is an excitatory amino acid transmitter that is found throughout the central nervous system. Glutamate receptors are divided into two major classes, ionotropic glutamate receptors and metabotropic glutamate receptors (mGluRs). Ionotropic glutamate receptors are ligand-gated ion channels. They can be classified into three types named for their most selective agonists, N-methyl-D-aspartic acid, kainic acid, and D,L-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA). All of these ionotropic glutamate receptor types are antagonized by kynurenic acid (3). In contrast to the ionotropic glutamate receptors, the mGluRs are G protein-coupled receptors that modulate second-messenger systems (7). To date, eight different mGluRs have been identified, and they can be categorized into three groups based on their amino acid sequence identity. The mGluRs can be activated independent of ionotropic glutamate receptors by 1S,3R-aminoacyclopentine-1,3-dicarboxylic acid (ACPD) and can be antagonized by α-methyl-4-carboxyphenylglycine (MCPG) (7).

Microinjection of glutamate into the NTS mimics activation of the baroreflex and produces a decrease in arterial pressure and heart rate (17–20, 22, 26). Both ionotropic glutamate receptors and mGluRs are present in the NTS (6, 13, 22), and both classes of receptors may play a role in the response to glutamate. Blockade of ionotropic glutamate receptors by injection of kynurenic acid into the NTS abolishes the arterial baroreflex (18–20, 22, 26). However, it has been reported that kynurenic acid does not attenuate the effects of exogenous glutamate injected into the NTS (20, 22, 26). One potential explanation for these data is that exogenous glutamate acts at a class of receptors that is insensitive to kynurenic acid (22, 26). Because activation of mGluRs in the NTS with ACPD produces depressor responses that are unaffected by kynurenic acid, it has been suggested (22) that mGluRs could mediate the effects of glutamate after ionotropic receptor blockade. Consistent with a depressor response, activation of mGluRs has been demonstrated to produce several excitatory, postsynaptic effects on NTS neurons. These effects include direct depolarization of neurons (9), facilitation of the effects of ionotropic glutamate receptor activation (10), and inhibition of the effects of GABA receptor activation (10). Taken together, these data suggest that, in addition to ionotropic glutamate receptors, exogenous glutamate injected into the NTS acts at mGluRs to produce decreases in arterial pressure and bradycardia. Thus the purpose of this study was to determine whether combined blockade of ionotropic glutamate receptors and mGluRs in the NTS blocks the effects of glutamate microinjection. This study extends previous work from other laboratories (22, 26) and directly tests the hypothesis that mGluRs, in addition to the ionotropic glutamate receptors, mediate the cardiovascular effects of glutamate in the NTS.

METHODS

Experimental preparation. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Missouri, Columbia. Adult, male Sprague-Dawley rats (n = 26) weighing 336 ± 10 g were anesthetized with urethan (1.2–1.5 g/kg ip). The right femoral artery and vein were cannulated (PE-10 fused to PE-50) for measurement of arterial pressure and administration of drugs, respec-
respectively. The arterial catheter was connected to a pressure transducer to record arterial pressure. Mean arterial pressure (MAP) was derived electronically using a low-pass filter. Heart rate (HR) was determined with a cardiotachometer triggered from the arterial pressure pulse. The trachea was cannulated, and the rats were ventilated artificially with O2-enriched room air. Rectal temperature was monitored and maintained within normal limits with a circulating water heating blanket. Electrodes for recording lumbar sympathetic nerve activity (LSNA) were implanted by a modification of a technique previously described (27). Through a midline abdominal incision, the lumbar sympathetic chain was identified immediately caudal to the left renal vein. Two Teflon-insulated, silver-wire electrodes (Medwire, 0.005 in. diameter, 36 gauge) threaded through Silastic tubing (0.025 in. ID) were placed around the isolated sympathetic chain. Nerves and electrodes were covered with a polyvinylsiloxane gel (Coltene President), which was allowed to harden before closure. A ground wire was attached to the exterior skin. The preparation was contained within a Faraday cage to help reduce electrical noise. Sympathetic nerve activity was amplified 1,000 times using a Grass preamplifier (P511) and filtered using a high-pass frequency level of 30 Hz and a low-pass frequency level of 3 kHz. Action potentials were rectified and integrated using a root mean square converter with a time constant of 28 ms. The rectified, integrated signal was then electronically averaged. Background noise was defined as the residual signal from the nerve after the animal was euthanized. LSNA was defined as the amount of recorded nerve activity after subtraction of background noise.

Microinjections. Rats were placed in a Kopf stereotaxic frame, and the dorsal surface of the medulla was exposed surgically via an approach through the atlanto-occipital membrane. Multibarrel pipettes (3, 5, or 7 barrels, outside tip diameter 65 ± 6 μm) were placed unilaterally into the NTS under visual guidance using a Storz surgical microscope. Target stereotaxic coordinates for the NTS were +0.5 mm rostral and ±0.5 mm lateral to the caudal tip of the area postrema (calamus scriptorius) and 0.5 mm ventral from the dorsal surface of the medulla. The criteria for accurate pipette placement within the NTS were depressor and sympathoinhibitory responses to pressure injection of 10 mM glutamate. If these criteria were not met, coordinates were adjusted, and the response to glutamate was retested. The average stereotaxic coordinates for pipette placement were +0.50 ± 0.03 mm rostral and ±0.50 ± 0.04 mm lateral to calamus scriptorius and 0.50 ± 0.00 mm ventral from the dorsal surface of the medulla. Drugs were ejected from the pipette in volumes of 30 nl over a period of <3 s by applying pulses of pressurized N2 to each barrel using a custom-constructed pressure ejection system. The volume of drug delivery was controlled by changing the injection pressure and/or duration of the pressure pulse. The volume of the injection was determined by viewing the movement of the fluid meniscus in individual barrels of known internal diameter using a microscope (×150) equipped with a calibrated eyepiece micrometer.

Experimental protocols. Preliminary studies with the mGlur antagonist MCPG were performed to determine a protocol that would effectively block mGlurRs within the NTS. The concentration of MCPG used in this study was selected on the basis of previously published pharmacological studies (11, 16). Blockade of mGlurRs was verified by inhibition of the cardiovascular effects of the mGlur agonist ACPD. Single injections of MCPG did not abolish the response to ACPD (Table 1). However, administration of 10 mM MCPG for 1 min by repeated injections of 30 nl every 10 s totally inhibited the effects of 1 mM ACPD, and these responses recovered within 2–5 min. The cardiovascular response to ACPD after the multiple injections of MCPG was not different from the response to a single injection of saline. Taken together, these data suggest that exposure to MCPG for 1 min via multiple injections into the NTS can be utilized to block mGlurRs.

To eliminate differences in administration of the antagonists, the ionotropic glutamate receptor antagonist kynurenic acid was administered in the same manner. To determine whether kynurenic acid given as repeated injections or as a single injection produced similar effects on the response to glutamate, studies were conducted that evaluated the response to glutamate before and after NTS administration of kynurenic acid using both protocols. Importantly, kynurenic acid produced qualitatively similar attenuation of a subsequent glutamate response regardless of the method of administration.

For all experiments, the general protocol was as follows. Control responses to unilateral microinjection of 30 nl glutamate (10 mM) were obtained. From a different pipette barrel, a glutamate antagonist was then injected into the NTS as described above. Immediately at the end of the 1-min period of antagonist injections, the glutamate injection was repeated. In addition, glutamate injections were repeated every 2–3 min after termination of the antagonist injections to test for recovery from blockade. A minimum of 2 min between sequential injections of glutamate was used to allow baseline parameters to return to preinjection levels. Preliminary studies demonstrated that 2 min between glutamate injections was adequate to prevent tachyphylaxis. A minimum of 45 and 15 min was allowed for recovery after injection of kynurenic acid and MCPG, respectively, before initiation of another experimental protocol. Throughout this study, the

Table 1. Peak responses to microinjection of 1 mM ACPD into the NTS before and after NTS administration of 10 mM MCPG

<table>
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<tr>
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<th>Peak ACPD Response</th>
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<th>Peak ACPD Response</th>
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<tr>
<td></td>
<td>Control</td>
<td>After MCPG: single injection</td>
<td>Control</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>−27 ± 4</td>
<td>−19 ± 4</td>
<td>−25 ± 4</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>−28 ± 2</td>
<td>−19 ± 5</td>
<td>−24 ± 7</td>
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<tr>
<td>LSNA, %control</td>
<td>−23 ± 3</td>
<td>−15 ± 2</td>
<td>−17 ± 3†</td>
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Values are means ± SE; n = 5. NTS, nucleus of the solitary tract; ACPD, 1S,3R-amino-4-cyclopentane-1,3-dicarboxylic acid; MCPG, α-methyl-4-carboxyphenylglycine; Δ, change; MAP, mean arterial pressure; HR, heart rate. LSNA, lumbar sympathetic nerve activity. *P < 0.05 compared with control agonist responses produced before MCPG administration and with the 9 min response after MCPG. †P < 0.05 compared with control agonist responses produced before MCPG administration.
concentrations of individual drugs were constant as follows: 10 mM glutamate, 1 mM ACPD, 40 mM kynurenic acid, and 10 mM MCPG.

Three different experiments were performed with NTS administration of glutamate antagonists. These experiments consisted of evaluation of responses to glutamate in the NTS before and after injections of kynurenic acid alone, MCPG alone, or the combination of kynurenic acid and MCPG. In the experiments using injection of both kynurenic acid and MCPG, different pipette barrels were used for each drug and, typically, the time of injection for each antagonist was staggered so that only one drug was being delivered at a time. To control for pressure and volume effects of the injections of antagonist, the effects of saline injections on glutamate responses were performed. Saline injections were administered as two 30-nl injections given every 10 s for 1 min. At the end of the experiment, rats were euthanized with an overdose of urethan administered through a venous catheter.

Histological analysis. In addition to functional identification by depressor and sympathoinhibitory responses to glutamate or ACPD injection, histological analysis was performed in some rats to confirm accurate pipette location within the NTS. At the end of the experiment, 30- nl of 2.5% Pontamine sky blue dye were ejected from a different pipette barrel to mark the injection site. After euthanasia, the brains were removed and stored in 10% phosphate-buffered Formalin that contained sucrose. Frozen 40-µm coronal sections were made of the medulla for localization of the dye spot.

Drugs. Monosodium L-glutamate, kynurenic acid, urethan, and Pontamine sky blue were obtained from Sigma Chemical (St. Louis, MO). ACPD was obtained from Research Biochemicals International (Natick, MA). MCPG was obtained from Tocris Cookson (St. Louis, MO). Drugs were dissolved in distilled water or 0.9% saline solution. Kynurenic acid and MCPG were first solubilized with 1 eq NaOH before dilution with vehicle and final pH adjustment to 7.2-7.6. Drug doses are expressed as the free base of each drug.

Data analysis. The LSNA responses to agonist injections were analyzed as a percentage of the control level of LSNA before control glutamate or antagonist injections. The control level of LSNA was defined to be 100%.

The percent inhibition of the different antagonists on the MAP, HR, and LSNA response to glutamate injection was calculated with the following formula: [(Con – Glux)/Con] × 100, where Con is the peak response to glutamate before antagonist administration and Glux is the peak response to glutamate after antagonist administration.

All data are expressed as means ± SE. Data comparing levels of MAP, HR, or LSNA before and after agonist injections during control, antagonist administration, and recovery were analyzed by two-way ANOVA with repeated measures. Peak changes in MAP, HR, or LSNA in response to agonist injections during control, antagonist administration, and recovery were analyzed by one-way ANOVA with repeated measures. In addition, the effects of antagonist injections on baseline parameters were analyzed by one-way ANOVA with repeated measures. When ANOVA indicated a significant interaction, differences between individual means were assessed by a least-significant difference (LSD) test (24). The peak changes in MAP, HR, or LSNA produced by glutamate injection after antagonist were compared with peak changes produced by vehicle injection by independent Student’s t-tests. A probability of P < 0.05 was considered statistically significant. All statistical analyses were performed using Sigma Stat for Windows (Jandel Scientific, San Rafael, CA) software package.

RESULTS

In all NTS injection sites, injection of glutamate or ACPD produced decreases in MAP, HR, and LSNA. In contrast to glutamate and ACPD, injection of 30 nl of saline (n = 5) into the NTS as a volume pressure control did not alter MAP [change (Δ) = 1 ± 1 mmHg], HR (Δ = 2 ± 2 beats/min), or LSNA (Δ = 1 ± 1% control). Histological analysis of the injection sites marked with dye (n = 12) verified that the pipettes were within the NTS. All of the pipette locations were within the intermediate NTS lateral to the area postrema ~500 µm rostral to calamus scriptorius (Fig. 1).

Effect of blockade of mGluRs. Baseline hemodynamic parameters and the effects of MCPG on MAP, HR, and LSNA are presented in Table 2. Unilateral NTS blockade of mGluRs did not alter MAP, HR, or LSNA from control values after 1 min of injection of MCPG nor during 20 min of recovery from MCPG injection.

The effect of blockade of mGluRs on the response to exogenous glutamate in the NTS in a single animal is illustrated in Fig. 2A, and average (n = 11) peak responses to glutamate before and after MCPG are presented in Fig. 3. Control injections of glutamate produced a decrease in MAP (−21 ± 2 mmHg), HR (−21 ± 5 beats/min), and LSNA (−30 ± 5% control). The peak MAP, HR, and LSNA response to glutamate was not altered after 1 min of MCPG administration nor at 2 min after the MCPG injections were stopped.
To control for nonspecific pressure and volume effects of the antagonist injections, experiments evaluating the effects of saline injections (given as two 30-nl injections every 10 s for 1 min) on the response to glutamate were performed. Saline injections, administered using the same injection volumes and protocol as the experiments using the combination of MCPG and kynurenic acid, did not alter the response to injection of glutamate \( (n = 5) \). A representative saline control experiment is presented in Fig. 2D.

Effect of blockade of ionotropic glutamate receptors. Baseline hemodynamic parameters and the effects of kynurenic acid on MAP, HR, and LSNA are presented in Table 2. None of these parameters were altered before the glutamate injections. Unilateral NTS blockade of ionotropic glutamate receptors in the NTS tended to increase MAP by 2 and 10 min and significantly elevated LSNA at 10 and 20 min after the kynurenic acid injections (Table 2). By 40 min, LSNA had returned to control levels.

Control injections of glutamate produced a decrease in MAP \( (-26 \pm 3 \text{ mmHg}) \), HR \( (-25 \pm 8 \text{ beats/min}) \), and LSNA \( (-28 \pm 6\% \text{ control}) \) (Figs. 2B and 4). After 1-min application of kynurenic acid, the peak MAP, HR, and LSNA responses to glutamate were attenuated; however, glutamate still produced significant decreases in MAP, HR, and LSNA \( (n = 10) \). Two minutes after the end of the kynurenic acid injections, the peak HR response to glutamate remained attenuated, although the MAP and LSNA responses were no longer different from control. All glutamate responses had recovered within 30 min of termination of the kynurenic acid administration.

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<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>LSNA, %control</th>
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<tr>
<td><strong>MCPG ( (n = 11) )</strong></td>
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<tr>
<td>Control</td>
<td>82 ± 7</td>
<td>393 ± 32</td>
<td>119 ± 7*</td>
</tr>
<tr>
<td>0 min</td>
<td>90 ± 8</td>
<td>379 ± 32</td>
<td>119 ± 7*</td>
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<tr>
<td>2 min</td>
<td>100</td>
<td>397 ± 34</td>
<td>114 ± 6*</td>
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<td>20 min</td>
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<td><strong>Kyn ( (n = 10) )</strong></td>
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<tr>
<td>Control</td>
<td>89 ± 4</td>
<td>361 ± 18</td>
<td>97 ± 3</td>
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<tr>
<td>0 min</td>
<td>101 ± 6</td>
<td>371 ± 19</td>
<td>117 ± 5*</td>
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<td>20 min</td>
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<tr>
<td><strong>MCPG + Kyn ( (n = 8) )</strong></td>
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<tr>
<td>Control</td>
<td>82 ± 7</td>
<td>393 ± 32</td>
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Values are means ± SE. Kyn, kynurenic acid. *\( P < 0.05 \) compared with control value before drug administration.

To control for nonspecific pressure and volume effects of the antagonist injections, experiments evaluating the effects of saline injections (given as two 30-nl injections every 10 s for 1 min) on the response to glutamate were performed. Saline injections, administered using the same injection volumes and protocol as the experiments using the combination of MCPG and kynurenic acid, did not alter the response to injection of glutamate \( (n = 5) \). A representative saline control experiment is presented in Fig. 2D.

**Fig. 2.** Examples from individual urethan-anesthetized rats of mean arterial pressure (MAP) and lumbar sympathetic nerve activity (LSNA) responses to 30-nl microinjection of 10 mM glutamate into the NTS before (left) and after (right) administration of different glutamate antagonists or vehicle into the NTS. A: 10 mM \( \alpha \)-methyl-4-carboxyphenylglycine (MCPG). B: 40 mM kynurenic acid (Kyn). C: combined 10 mM MCPG and 40 mM kynurenic acid. D: saline.
In addition to testing the effect of 1-min exposure of kynurenic acid on the glutamate responses, the effect of a single injection (67 ± 8 nl) of kynurenic acid on the response to glutamate was evaluated (n = 10). A single injection of kynurenic acid produced a qualitatively similar attenuation of the response to glutamate (Table 3) compared with the reduction of the glutamate response when kynurenic acid was administered for 1 min (Fig. 4). The depressor effect of glutamate tended to be reduced by 1 min after injection of kynurenic acid, while the sympathoinhibitory and bradycardic responses to injection of glutamate were significantly blunted. The effects of kynurenic acid on the sympathoinhibitory response to glutamate but not the bradycardia had recovered within 9 min. In these experiments, administration of kynurenic acid either as single or multiple injections attenuated but did not abolish the response to injection of glutamate.

The effect of combined blockade of ionotropic glutamate receptors and mGlurRs. Baseline hemodynamic parameters and the effects of combined kynurenic acid and MCPG on MAP, HR, and LSNA are presented in Table 2. None of these parameters was altered before the glutamate injections. Unilateral NTS combined blockade of ionotropic glutamate receptors and mGlurRs tended to increase MAP and LSNA by 2 min, and LSNA was significantly elevated by 10 min after the antagonist injections (Table 2). By 40 min, MAP, HR, and LSNA had returned to control levels.

Control injections of glutamate produced decreases in MAP (−19 ± 3 mmHg), HR (−13 ± 6 beats/min), and LSNA (−25 ± 3% control) (Figs. 2C and 5). The sympathoinhibitory response to microinjection of glutamate...
mate was abolished and the decrease in MAP was markedly attenuated after combined administration of kynurenic acid and MCPG (n = 8). There was a tendency for the HR response to be attenuated, although this did not reach statistical significance. Importantly, the MAP, HR, and LSNA responses to glutamate after combined injection of kynurenic acid and MCPG were not different from the responses to injection of 30 nl of saline. This finding suggests that the responses to glutamate after kynurenic acid and MCPG were reduced to the effect of microinjection, and therefore the effects of glutamate were blocked. Two minutes after the antagonist injections were stopped, the peak MAP response to glutamate recovered and was similar to the control glutamate response. The LSNA response to glutamate at this time had significantly recovered but was still attenuated compared with control. All responses had recovered within 30 min of termination of the combined kynurenic acid and MCPG administration.

To compare the attenuation of the glutamate response among MCPG alone, kynurenic acid alone, and MCPG and kynurenic acid together, the percent inhibition of the glutamate response by the antagonists was calculated and is presented in Fig. 6. The percent inhibition of the glutamate response was calculated for MAP, HR, and LSNA. Administration of MCPG alone did not significantly alter the response to injection of glutamate (Fig. 3), and MCPG alone produced minimal percent inhibition of the response to glutamate (Fig. 6). Exposure to kynurenic acid alone significantly reduced the response to injection of glutamate (Fig. 4) and produced a moderate level of percent inhibition, although the percent inhibition was not significantly different between kynurenic acid alone and MCPG alone (Fig. 6). In contrast, combined administration of MCPG and kynurenic acid abolished the sympathoinhibition and markedly attenuated the depressor response to glutamate (Fig. 5). The percent inhibition of the HR effects of glutamate was not different among the three groups. However, the percent inhibition of the depressor and sympathoinhibitory response to glutamate was greater after combined administration of MCPG and kynurenic acid compared with either MCPG or kynurenic acid alone (Fig. 6). Thus the reduction in the response to injection of glutamate was greater by combination of MCPG and kynurenic acid than by either antagonist alone.

**DISCUSSION**

This study tested the hypothesis that glutamate microinjected into the NTS activates both ionotropic glutamate receptors and mGluRs to produce its cardiovascular effects. Changes in MAP, HR, and LSNA were recorded during glutamate injections into the NTS before and after NTS administration of selective glutamate receptor antagonists. The major finding of this study was that the cardiovascular response to glutamate injection was blocked by combined antagonism of ionotropic glutamate receptors and mGluRs. This effect of combined administration of kynurenic acid and MCPG on the response to glutamate was significantly greater than the effect of either antagonist alone. These
glutamate were blocked if the mGluR antagonist, MCPG, was combined with kynurenic acid. These data suggest that, after ionotropic glutamate receptor blockade, the response to glutamate was mediated by mGluRs. A role for mGluRs in the NTS is also suggested by the fact that activation of these receptors with the mGluR agonist ACPD mimicked the response to glutamate. These data support the conclusion that exogenous glutamate activates mGluRs in the NTS to produce depressor, bradycardic, and sympathoinhibitory responses.

In light of these findings, it is somewhat surprising that blockade of mGluRs alone with MCPG did not alter the response to glutamate. In this study, a relatively high dose of glutamate (300 pmol in 30 nl) was utilized. On the basis of NTS dose-response curves generated in similar preparations in other laboratories, this dose is within or near the upper plateau of the dose-response curve (20, 26). Our data suggest that glutamate acts at both ionotropic glutamate receptors and mGluRs to produce neuronal activation. However, at this dose, glutamate may produce near-maximal levels of neuronal activation via stimulation of ionotropic glutamate receptors alone. In this situation, blockade of mGluRs alone would not alter the glutamate response, and the effects of glutamate at mGluRs would only be seen after ionotropic receptor blockade. The greater effect of activation of ionotropic glutamate receptors could be due to a variety of factors, including the relative effectiveness of these receptors to activate neurons or differences in receptor number, location, or affinity for glutamate.

In this study, the combination of kynurenic acid and MCPG abolished the sympathoinhibitory effect of glutamate microinjection. However, small residual depressor and bradycardic responses to glutamate were present. There are several possible explanations for this difference in the effects of the antagonists on the sympathoinhibitory and depressor responses to glutamate. One possibility is that combined blockade with kynurenic acid and MCPG may not completely block all of the glutamate receptors. Although MCPG has been described and utilized as an effective mGluR antagonist, the drug has a low potency (7, 16, 23). In addition, MCPG does not effectively block all mGluRs nor does it block all mGluRs with equal potency (7, 23). Thus it is possible that the small residual response to glutamate after combined kynurenic acid and MCPG administration is due to activation of a subtype of mGluR that is less sensitive to MCPG. In addition, although there is no direct evidence, there may be distinct neurons in the NTS that primarily influence LSNA, other sympathetic nerves (e.g., renal or splanchnic), or HR (parasympathetic or sympathetic). If mGluRs are differentially expressed and utilized as an effective mGluR agonist ACPD mimicked the response to glutamate. These data support the conclusion that exogenous glutamate activates mGluRs in the NTS to produce depressor, bradycardic, and sympathoinhibitory responses.

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In this study, the combination of kynurenic acid and MCPG abolished the sympathoinhibitory effect of glutamate microinjection. However, small residual depressor and bradycardic responses to glutamate were present. There are several possible explanations for this difference in the effects of the antagonists on the sympathoinhibitory and depressor responses to glutamate. One possibility is that combined blockade with kynurenic acid and MCPG may not completely block all of the glutamate receptors. Although MCPG has been described and utilized as an effective mGluR antagonist, the drug has a low potency (7, 16, 23). In addition, MCPG does not effectively block all mGluRs nor does it block all mGluRs with equal potency (7, 23). Thus it is possible that the small residual response to glutamate after combined kynurenic acid and MCPG administration is due to activation of a subtype of mGluR that is less sensitive to MCPG. In addition, although there is no direct evidence, there may be distinct neurons in the NTS that primarily influence LSNA, other sympathetic nerves (e.g., renal or splanchnic), or HR (parasympathetic or sympathetic). If mGluRs are differentially expressed and utilized as an effective mGluR agonist ACPD mimicked the response to glutamate. These data support the conclusion that exogenous glutamate activates mGluRs in the NTS to produce depressor, bradycardic, and sympathoinhibitory responses.
Over the past decade, there has been substantial interest in the hypothesis that glutamate is the neurotransmitter for arterial baroreceptor afferents that terminate in the NTS (1, 12, 25). One controversial issue related to this hypothesis has been the effect of kynurenic acid on the response to microinjection of glutamate into the NTS. Some studies have reported kynurenic acid to reduce (18) or abolish (19) the effects of glutamate injected into the NTS. Other investigators reported no attenuation or even an enhancement of glutamate responses by pretreatment with kynurenic acid (20, 22, 26). In the current study, kynurenic acid reduced but did not abolish the cardiovascular effects of glutamate. The reason for the differences in the ability of kynurenic acid to affect the glutamate response among these studies is not readily apparent. The important point of the current data and the majority of the previously published reports is that blockade of ionotropic glutamate receptors alone with kynurenic acid did not abolish the glutamate response. This suggests that the response to microinjection of glutamate is not totally dependent on ionotropic glutamate receptors. Thus, as suggested by others (22, 26), it appears that exogenous glutamate in the NTS may act at a class of kynurenic acid-insensitive receptors. The data from the present study confirm that the mGluRs are this class of kynurenic acid-insensitive receptors.

Several effects of activation of mGluRs within the NTS have been identified, and the majority of these responses would be defined as modulatory. In the NTS brain slice preparation, activation of mGluRs produces both excitatory and inhibitory responses (9, 10). In these studies, activation of mGluRs with ACPD produced direct depolarization of NTS neurons, facilitated AMPA currents, and inhibited GABA currents. These effects are postsynaptic and should produce or enhance neuronal excitability. Importantly, the present study and others (22) have demonstrated that activation of mGluRs within the NTS with exogenous ACPD produces cardiovascular responses similar to glutamate. These effects are consistent with excitatory responses of mGluRs on NTS neurons.

In addition to excitatory effects of mGluRs, activation of mGluRs in the NTS also appears to presynaptically inhibit glutamate release (9, 10). This conclusion is supported by the work of Hay and colleagues (14, 15), who demonstrated that activation of mGluRs inhibited calcium channels in nodose ganglion neurons and reduced vesicle exocytosis from identified aortic baroreceptor neurons. These mechanisms could contribute to the presynaptic inhibition of transmitter release that has been described. In the current study, an inhibitory effect of mGluR activation would be masked by the predominant excitatory responses elicited by microinjection of agonist. Studies that utilize selective agonists and antagonists to evaluate the endogenous and exogenous effects of different mGluRs will be helpful and necessary. In addition, studies investigating the effects of mGluRs on different reflex systems that relay in the NTS and in the integration of information at the level of the NTS are needed. The present study does not address these questions, but the data suggest that mGluRs are present functionally in the NTS and activation can produce robust cardiovascular responses.

Throughout the central nervous system, activation of mGluRs produces several effects that can modulate neuronal function, including interactions with ionotropic glutamate receptors (4, 10). Furthermore, mGluRs appear to be involved in several forms of synaptic plasticity, including long-term potentiation and long-term depression (5, 21). Although no direct evidence has been presented, the presence of functional mGluRs within the NTS is compatible with the hypothesis that mGluRs modulate cardiovascular regulatory mechanisms. One possibility is that mGluRs tonically modulate arterial baroreflex function. Another possibility is that mGluRs are involved in the modulation of arterial baroreflex function that occurs during changes in physiological and/or pathophysiological states. Effects of mGluRs that are excitatory could produce leftward and/or downward shifts in arterial baroreflex function such as seen with elevated circulating levels of vasopressin. In contrast, inhibitory effects of mGluRs would blunt and shift the arterial baroreflex curve to the right and could contribute to changes in reflex function due to acute or chronic hypertensive resetting, stress, exercise, etc. At the level of the NTS, mGluRs have the potential to exert several important effects, including a role in cardiovascular reflex function and integration.

In summary, previous studies have documented that blockade of ionotropic glutamate receptors with kynurenic acid abolishes the arterial baroreflex but does not block the response to microinjection of glutamate. Activation of mGluRs with ACPD within the NTS produces effects qualitatively similar to glutamate. Importantly, the present study demonstrates that combined blockade of ionotropic glutamate receptors and mGluRs with kynurenic acid and MCPG blocks the depressor, bradycardic, and sympathoinhibitory response produced by glutamate injection into the NTS. These data suggest that exogenous glutamate microinjected into the NTS acts at both ionotropic glutamate receptors and mGluRs. Blockade of both classes of glutamate receptors is required to block the cardiovascular response to microinjection of glutamate in the NTS. These data are consistent with the concept that mGluRs in the NTS may be involved in the modulation and/or regulation of cardiovascular function.

 Perspectives

Previous studies have shown that kynurenic acid administration into the NTS eliminates arterial baroreflex function but does not block the response to microinjection of glutamate. In the current study, combined application of kynurenic acid and MCPG was necessary to effectively block the response to exogenous glutamate. There are several possibilities that could explain the difference in the ability of kynurenic acid to antagonize endogenous glutamate released from baroreceptor afferents and exogenous glutamate microinjected into the NTS. The NTS is a heterogenous population of neurons and contains neurons that are involved in a variety of pathways. One possibility is that ionotropic...
glutamate receptors are required for normal arterial baroreflex function, whereas mGluRs are involved in other pathways that are integrated in the NTS and that modulate baroreflex function. This modulation could originate from descending pathways from other nuclei or from other afferent reflex systems. Therefore, although kynurenic acid blocks the arterial baroreflex, exogenous glutamate in the presence of kynurenic acid could activate these mGluRs to produce depressor and sympathoinhibitory responses independent of ionotropic glutamate receptors. Second, the arterial baroreflex may contain multiple glutamatergic synapses in series through the NTS, one of which is dependent on ionotropic glutamate receptors for transmission. In this case, blockade of ionotropic glutamate receptors would inhibit arterial baroreflex function by preventing neuronal transmission at the synapse that is void of mGluRs, but exogenous application of glutamate still could produce effects at distal neurons that contained mGluRs. Third, glutamatergic synapses within the NTS may contain mGluRs at extrasynaptic locations that are not readily accessible to endogenous glutamate. These extrasynaptic mGluRs may be involved in periods of high level of activation of baroreceptor afferents and increased transmitter release but not during brief changes in afferent activity. Exogenous glutamate could produce sympathoinhibition and depressor responses independent of ionotropic glutamate receptors by activating these extrasynaptic mGluRs to produce neuronal excitation.

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