Influence of GABA in the nucleus of the solitary tract on blood pressure in baroreceptor-denervated rats

Ito, Satoru, and Alan F. Sved. Influence of GABA in the nucleus of the solitary tract on blood pressure in baroreceptor-denervated rats. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1657–R1662, 1997.—We have previously reported that inhibition of the nucleus of the solitary tract (NTS) in chronically sinoaortic baroreceptor-denervated (SAD) rats has no effect on blood pressure in contrast to the marked increase in blood pressure it elicits in baroreceptor-intact rats. This could result either from a lack of tonic excitatory input to this region or from overriding inhibition of NTS neurons involved in the control of blood pressure. The present study aimed to distinguish between these two possibilities by examining the changes in blood pressure elicited by injection of bicuculline (Bic), a γ-aminobutyric acid (GABA) antagonist, into the NTS of SAD and control rats. In chloralose-anesthetized baroreceptor-intact rats or acutely SAD rats, injection of 10 pmol Bic into the NTS elicited minimal changes in blood pressure. In contrast, in chronic SAD rats injection of Bic into the NTS elicited a large decrease in blood pressure. The maximal decrease in blood pressure elicited by Bic in chronic SAD rats was equivalent to the maximal decrease in blood pressure that could be evoked by direct excitation of the NTS with L-glutamate. These results suggest that the lack of a tonic role of the NTS in the regulation of blood pressure in chronic SAD rats is a result of maximal GABA-mediated inhibition of relevant NTS neurons.

Methods

Animals. Adult male Sprague-Dawley rats (Zivic-Miller Laboratories, Zeilienople, PA) were individually housed in a temperature-controlled room (22–23°C) on a 12:12-h light-dark cycle. Tap water and Purina Chow pellets were available ad libitum.

Sinoaortic denervation. Sinoaortic denervation was performed as described previously (10). Rats were anesthetized with halothane, and the region of the carotid bifurcation was exposed. With the use of a surgical microscope, the carotid depressor nerve, superior cervical chain, and superior laryngeal nerve were transected. The common carotid artery, carotid bifurcation, and branches of the internal and external carotid arteries were stripped of all visible nerve fibers and wiped with 10% phenol in ethanol. These procedures were performed bilaterally, and then the neck wound was closed with wound clips. Rats were allowed to recover for 10–14 days. During each of the first few days after sinoaortic denervation, rats were given 10 ml of saline subcutaneously. Control rats were not subjected to any surgical manipulation.

Baroreceptor reflex testing. On the day before the experiment, rats were anesthetized with halothane and arterial and venous femoral catheters were inserted, with the ends tunneled subcutaneously to exit between the scapulae. Each rat was fitted with a jacket connected to a tether and swivel system that allowed free movement of the rat. Rats were returned to their cage and allowed to recover overnight. Arterial baroreceptor reflexes were tested in all rats by intravenous injections of phenylephrine (2 µg/kg) and nitroprusside (5 µg/kg) (11). In control rats, phenylephrine elicits an increase in MAP of ~45 mmHg and a reflexive bradycardia of ~65 beats/min (bpm); nitroprusside decreases MAP ~30 mmHg and increases heart rate (HR) ~65 bpm (11). Of the 23 rats subjected to the sinoaortic denervation surgery for this study, 6 rats had no detectable reflex changes in HR in response to either drug and were therefore considered to be effectively denervated (11); these are referred to as SAD rats or complete SAD rats. The remaining rats had some degree of residual reflexive HR response to at least one of the drugs and were classified as near total SAD rats if reflex changes in HR did not exceed 25 bpm (n = 7) or partial SAD rats if reflex changes in HR to either drug exceeded 25 bpm (n = 10).

Microinjection protocols. After completion of baroreceptor reflex testing, anesthesia was induced with halothane. The present study sought to distinguish between these two possibilities. On the basis of the expectation that in SAD rats cardiopulmonary receptors may continue to provide tonic excitatory input to the NTS, it was our hypothesis that the lack of an effect of muscimol injected into the NTS on MAP in chronic SAD rats is due to active inhibition of the NTS neurons rather than a lack of tonic excitatory input. This hypothesis leads to the testable prediction that, in chronic SAD rats, blockade of GABA-mediated neuronal inhibition in the NTS should elicit a marked decrease in MAP by unmasking tonically active excitatory input to NTS neurons involved in cardiovascular control.

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trachea was cannulated and the rat was artificially ventilated with 100% oxygen containing halothane (~2%). Chloralose (60 mg/kg iv) and tubocurarine (0.5 mg/kg iv) were then administered and the halothane was terminated. Supplemental doses of chloralose (20 mg/kg) and tubocurarine (0.2 mg/kg) were administered every hour; experiments did not last >3 h. The rat was placed in a stereotaxic frame and the dorsal surface of the medulla was surgically exposed. Microinjections were made into the NTS (coordinates: 0.5 mm rostral to the caudal tip of the area postrema, 0.5 mm lateral, 0.5 mm below the medullary surface) using single-barrel glass micro-pipettes and a PicoPump. All microinjections were made in a volume of 100 nl of artificial cerebrospinal fluid administered over several seconds. In experiments requiring multiple injections, the pipette was withdrawn, refilled, and reinserted at the same coordinates. Subsequent injections were made at least 15 min after the response to the previous injection had ended. The drugs injected into the NTS were nipecotic acid (Sigma Chemicals), glutamate monosodium (Sigma Chemicals), and bicuculline methiodide (Bic; Research Biochemicals International).

The protocol of the initial experiment was as follows. First, glutamate (200 pmol) was injected into the right NTS, followed at 15-min intervals with the additional doses of glutamate. Then, the dose-response relationship for glutamate injected into the NTS was tested on the left side. Fifteen minutes later, the effect of bilateral injection of nipecotic acid into the NTS was tested; this indirect-acting GABA agonist was injected bilaterally rather than unilaterally, because we have previously shown that neuroinhibitory drugs need to be injected bilaterally to elicit a pressor response. At least 60 min after injection of nipecotic acid, the effects of injection of Bic into the right NTS were tested. This was followed at least 30 min later by injection of Bic into the contralateral NTS. Thus, responses to unilateral injection of Bic and glutamate were tested in each rat. For these drugs, data for injection into the right NTS are presented. There were no significant differences between responses elicited from the left and right sides (P > 0.05 for Bic and glutamate). In the experiment in which the effect of vagotomy on the response to Bic was examined, rats received only injections of Bic into the NTS.

Acute sinoaortic denervation. A group of acutely SAD rats were anesthetized with halothane and sinoaortic denervation was performed as described above. After completion of the denervation, chloralose (60 mg/kg iv) was administered and the halothane was terminated. Rats remained anesthetized and were studied beginning 2 h later in the microinjection protocols, as described above.

Histological analysis of injection sites. At the conclusion of most experiments, 100 nl of 1% fast green dye was injected into the NTS to mark the site of injection. After death of the rat by overdose of anesthetic, the brain was removed and frozen. The brain stem was then sectioned (40 µm) using a cryostat/microtome, and brain sections were thaw-mounted onto glass microscope slides. After staining of the brain sections with cresyl violet, the microinjection site was examined using low-magnification light microscopy. All microinjection sites were centered in the medial subnucleus of the NTS adjacent to the area postrema.

Data analysis. Data are presented as means ± SE. Comparisons between groups were done using one-way analysis of variance (ANOVA), with post hoc comparisons using the Tukey-Kramer test. Comparisons between drugs or drug doses and groups were done using two-way repeated-measures ANOVA.

RESULTS

Baseline MAP of chronic SAD rats anesthetized with chloralose (104 ± 3 mmHg; n = 6) was not different from MAP in control, baroreceptor-intact rats (103 ± 2 mmHg, n = 8). Similarly baseline HR were similar in SAD and control rats (380 ± 20 and 341 ± 8 bpm, respectively; P > 0.05). In baroreceptor-intact rats, bilateral injection into the NTS of the indirect-acting GABA agonist nipecotic acid (10 nmol) increased MAP by 42 ± 4 mmHg, consistent with previous observations (3). In contrast, nipecotic acid had no effect on blood pressure in each of the six chronic SAD rats (0 ± 0 mmHg), consistent with our previous observations with other drugs that act to inhibit NTS neurons (10). A group of rats that underwent the SAD surgery, but still had marked baroreceptor reflex responses evoked by phenylephrine and nitroprusside (partial SAD, change of HR of >25 bpm to either phenylephrine or nitroprusside) responded to nipecotic acid similar to control rats (31 ± 9 mmHg, n = 10; P > 0.05 compared with control). Rats that underwent the SAD surgery and had nearly complete loss of baroreceptor reflex responses (near total SAD, change of HR <25 bpm to both phenylephrine and nitroprusside) still responded to nipecotic acid injected into the NTS (25 ± 12 mmHg; n = 7), although because of the variability of this response it was not statistically different from either control rats or chronic SAD rats. Baseline MAP and HR in the groups of rats labeled partial SAD or near total SAD were not different from the control and complete SAD groups.

Unilateral injection of Bic (10 pmol) into the NTS of control rats did not significantly decrease MAP or HR (Figs. 1 and 2), consistent with previous data (3, 14). In marked contrast, injection of Bic into the NTS of SAD rats evoked a large depressor response along with bradycardia (Figs. 1 and 2). Responses elicited by injection of Bic into the NTS of partial and near-total SAD rats were intermediate between the responses evoked in control and SAD rats.
observed in control and complete SAD rats (Fig. 3). The magnitude of the Bic-evoked depressor response was inversely correlated with the largest change in HR during the testing of baroreceptor reflexes (correlation coefficient $= 0.76$, $P < 0.01$, $n = 23$).

Glutamate injected into the NTS produced dose-dependent decreases in MAP and HR in both complete SAD rats and control rats (Fig. 4). However, in SAD rats glutamate was more potent and effective in eliciting the depressor and bradycardic responses and the duration of the responses was greatly prolonged. Glutamate-evoked responses in partial SAD rats and near-total SAD rats were intermediate between control and baroreceptor-denervated rats (Fig. 4), and there was a significant inverse correlation of the glutamate-evoked depressor response and the baroreceptor-evoked change in HR (correlation coefficient $0.64$, $P < 0.05$, $n = 23$). In SAD rats, the maximal glutamate-evoked depressor response ($59 \pm 7$ mmHg) was similar to the response evoked by Bic ($52 \pm 5$ mmHg; $P > 0.05$). Interestingly, a similar relationship between the depressor response elicited by Bic and the maximal response elicited by glutamate was noted in the near-total SAD rats (Fig. 5).
To determine whether the large decrease in MAP elicited by injection of Bic into the NTS of chronic SAD rats was simply the result of a lack of baroreceptor reflexes, the effect of Bic injected into the NTS of acute SAD rats was examined. Chloralose-anesthetized rats studied 2–4 h after sinoaortic denervation had elevated MAP (135 ± 3 mmHg, n = 10, P < 0.05 compared with control). HR (376 ± 11 bpm) was also slightly elevated compared with control rats (P < 0.05). Injection of nipeptic acid into the NTS did not alter MAP or HR in these acute SAD rats. Bic injected into the NTS in acute SAD rats decreased MAP (−14 ± 3 mmHg); this response was markedly smaller than that observed in chronic SAD rats (Fig. 3). In contrast to the difference between Bic-evoked depressor responses in acute and chronic SAD rats, the depressor response elicited by a maximally effective dose of glutamate (200 pmol) was similar in these two groups of rats (Fig. 4).

To determine whether cardiopulmonary baroreceptor afferents contributed to the Bic-evoked decrease in MAP in chronic SAD rats, the effect of Bic injected into the NTS in a separate group of complete SAD rats was examined before and 1 h after bilateral cervical vagotomy. Vagotomy did not alter the depressor response elicited by injection of Bic into the NTS in chronic SAD rats (Table 1).

### DISCUSSION

The principal finding in these studies is that in chronic SAD rats blockade of GABA_A-mediated neuronal inhibition in the NTS by local injection of Bic elicits a marked vasodepressor and bradycardic response that is not observed in either baroreceptor-intact rats or acute SAD rats. We previously reported that inhibition or electrolytic lesion of the NTS in chronic SAD rats did not influence MAP (10). In contrast to the hypertensive response produced by inhibition of the NTS in baroreceptor-intact rats. Those results suggest that in chronic SAD rats the NTS does not provide any tonic influence on the neural control of the circulation. The present results indicate that this lack of a tonic effect of the NTS on MAP and HR is due not to the absence of tonic excitatory drive of NTS neurons involved in cardiovascular control, but rather the functional blocking of this input by GABA_A receptor-mediated neuronal inhibition.

The conclusion that enhanced GABA_A receptor-mediated neuronal inhibition in the NTS is responsible for the lack of tonic excitatory input of the NTS on cardiovascular control is based on the assumption that Bic acts selectively as a competitive antagonist of GABA_A receptors. We have previously documented that the dose of Bic used in the present study functionally antagonizes GABA_A receptors in the NTS without altering the function of GABA_B receptors in this region (14); similar data have been published by other investigators (8). Furthermore, injection of a GABA_B receptor antagonist into the NTS of SAD rats does not result in a large decrease in MAP (A. M. Schreihofer and A. F. Sved, unpublished observation).

The apparent upregulation of GABA_A receptor-mediated neuronal inhibition in the NTS of chronic SAD rats is not simply a reflection of the lack of baroreceptor buffering of evoked cardiovascular changes. Nonetheless, removal of baroreceptor reflexes does potentiate evoked cardiovascular responses, and this is evident in the potentiation of depressor responses elicited by injection of glutamate into the NTS. The notion that the larger glutamate-evoked responses in chronic SAD rats result solely from the elimination of baroreceptor-mediated cardiovascular compensations is supported by the similarity of glutamate-evoked responses in both acute and chronic SAD rats (Fig. 4). The similarity of glutamate-evoked responses between acute and chronic SAD rats stands in marked contrast to the difference in the response to Bic between these two groups of rats. These observations cannot be explained simply by loss of baroreceptor reflexes causing an exaggeration of all depressor responses.

The marked depressor and bradycardic response elicited by injection of Bic into the NTS of chronic SAD rats presumably results from the unmasking of a tonic excitatory input, because in the absence of an excitatory input it is unlikely that these neurons would fire action potentials. NTS neurons involved in cardiovascu-

### Table 1. Effect of acute vagotomy on the depressor response elicited by Bic in chronic SAD rats

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<td></td>
<td>Baseline</td>
<td>Change with Bic</td>
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<tr>
<td>Prevagotomy</td>
<td>121 ± 3</td>
<td>−51 ± 11</td>
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<tr>
<td>Postvagotomy</td>
<td>110 ± 6</td>
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Values are means ± SE. Effects of bicuculline (Bic) (10 pmol) injected into the nucleus of the solitary tract of chloralose-anesthetized chronic complete sinoaortic baroreceptor-denervated (SAD) rats (n = 3) were measured before bilateral cervical vagotomy and then again −1 h postvagotomy. Vagotomy did not significantly alter the response (P > 0.05, paired t-test). MAP, mean arterial pressure; HR, heart rate.
lar regulation are thought to receive the majority of their excitatory drive from baroreceptor afferent nerves, but in SAD rats this input has been eliminated. The excitatory drive of NTS neurons in chronic SAD rats is not provided by cardiopulmonary baroreceptors or other vagal afferent nerves, because vagotomy did not alter the response to injection of Bic into the NTS of chronic SAD rats. Thus, at present, the source of this excitatory input remains to be determined.

The difference in Bic-evoked depressor and bradycardic responses between acute (2 h) SAD rats and chronic (10–14 days) SAD rats suggests that the response takes some time to develop; at present, there are no additional data relating to this issue. Studies on other sensory afferent systems support the hypothesis that at least some of the compensations involving GABA_A-mediated neuronal inhibition may take many days to develop. Sciatic nerve section has been reported to lead to an increase in the number of GABA_A receptors in the projection field of these afferents in the dorsal horn of the spinal cord (1), a change that occurs between the first and second week after nerve section. Because this change in GABA_A receptors in the spinal cord in response to sciatic nerve section occurs along with a decrease in GABA level in the spinal cord (2), it is difficult to predict whether GABA_A receptor-mediated neuronal inhibition would be expected to be exaggerated or attenuated. Nonetheless, that sensory nerve fiber damage in these studies result in changes in GABA-mediated neural transmission that develop slowly is supported by these studies.

In addition to the completely baroreceptor-denervated chronic SAD rats examined in these studies, we included rats that had been subjected to the SAD surgery but still had residual baroreceptor reflexes. The Bic-evoked response was inversely related to degree of baroreceptor denervation, suggesting that the extent of exaggeration of GABA_A receptor-mediated neuronal inhibition is related to extent of baroreceptor reflex impairment produced by the SAD surgery. However, when the Bic-evoked response is considered in relation to the maximal glutamate-evoked depressor response obtained in each animal, a slightly different picture emerges. The response ratio of Bic to glutamate was similar across groups of SAD rats independent of the degree of baroreceptor denervation; this result would seem to support the conclusion that GABA receptor-mediated neuronal inhibition is enhanced in all groups, but that the Bic-induced depressor response is dampened by the baroreceptor reflex in animals that have residual reflex responses. However, if this were the case, then the NTS should not have a tonic influence on the cardiovascular system, and we have previously shown that in partial and near total SAD rats the NTS does provide tonic inhibition to influence cardiovascular control (10, 11). Even so, if remaining baroreceptor afferents provide a tonic excitatory signal to the NTS, the degree of GABA-mediated neuronal inhibition may be insufficient to block it completely.

The most straightforward interpretation of the results of the present study is that in response to chronic sinoaortic denervation there is an increase in GABA-mediated inhibition of the NTS. Furthermore, this input is sufficient to effectively eliminate a tonic role of the NTS in cardiovascular regulation. However, the nature of this GABAergic innervation and its regulation remain completely unknown. GABA interneurons are likely present within the NTS (12), but their role in cardiovascular regulation is uncertain. Input to the NTS, including input from the hypothalamus (7) and parabrachial nucleus (9), can inhibit barosensitive NTS neurons, but whether those responses are due to GABAergic afferents or input to GABAergic interneurons is unclear. Thus the source of this inhibitory drive, as well as its regulation, is unknown.

As noted above, Bic injected into the NTS of SAD rats, but not baroreceptor-intact rats, reduced MAP. This observation suggests that GABA is tonically active in the NTS in SAD rats but not in control rats. In contrast, the exactly opposite conclusion appears to be supported by the change in MAP elicited by injection of nipecotic acid into the NTS. However, this apparent conflict in the responses to these two drugs can be explained by actions on different pools of GABA receptors. We have previously documented that the pressor response elicited by injection of nipecotic acid into the NTS is mediated via an action at GABA_B receptors, not GABA_A receptors (12, 14). In chronic baroreceptor-denervated rats, this action of GABA on GABA_B receptors is not observed, possibly because of the prominent inhibitory effect of GABA mediated via GABA_A receptors.

In summary, the present data demonstrate that changes within the NTS act to dampen the tonic role of the NTS in cardiovascular regulation after baroreceptor denervation. The results suggest that GABA-mediated neural transmission in the NTS involved in cardiovascular regulation is capable of long-term change as a result of alterations in baroreceptor afferent input.

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