Rostral Ventrolateral Medulla Suppresses Reflex Bradycardia by the Release of Gamma-aminobutyric Acid in Nucleus Tractus Solitarii of the Rat

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KEY WORDS rostral ventrolateral medulla; nucleus tractus solitarii; reflex bradycardia; GABAergic neurotransmission; glutamate; in vivo microdialysis; rat

ABSTRACT We investigated the role of gamma-aminobutyric acid (GABA) in the nucleus tractus solitarii (NTS), the principal recipient of baroreceptor afferent fibers in the medulla oblongata, in the suppression of cardiac baroreceptor reflex (BRR) response by the rostral ventrolateral medulla (RVLM). Direct microinfusion via reverse microdialysis of L-glutamate (50 μM) into the RVLM promoted an inhibition of the BRR response, alongside an increase in the concentration of GABA in the dialysate collected from the ipsilateral NTS. Such an increase in GABA concentration in the NTS to RVLM activation was site-specific, as microinfusion of L-glutamate into areas outside the confines of RVLM resulted in no discernible change in GABA concentration in the dialysate of the NTS and minimal effect on the cardiac BRR response. The RVLM-induced BRR suppression of cardiac BRR response to microinjection into the bilateral RVLM of L-glutamate (1 nmol) was antagonized by administration into the bilateral NTS of the $GABA_A$ receptor antagonist, bicuculline methiodide (1 or 5 pmol), or the $GABA_B$ receptor antagonist, 2-hydroxy-saclofen (100 or 500 pmol). These results suggest that GABA released in the NTS may participate in cardiac BRR suppression induced by glutamatergic activation of the RVLM, via an action on both $GABA_A$ and $GABA_B$ receptor subtypes. **Synapse 39:23–31, 2001.**

INTRODUCTION

The rostral ventrolateral medulla (RVLM) plays a pivotal role in central regulation of blood pressure (Guyenet, 1990; Reis et al., 1994; Spyer, 1994, for review). As the sympathetic premotor neurons in the medulla oblongata, RVLM is the origin of bulbospinal afferents to the sympathetic preganglionic neurons in the spinal cord (Ross et al., 1984; Guyenet, 1990). In addition to providing a tonic modulation of the sympathetic vasomotor activity of blood vessels (Guyenet, 1990; Reis et al., 1994), it also participates in baroreceptor reflex (BRR) control of systemic arterial pressure (Ross et al., 1984; Spyer, 1994). As the primary terminal site of baroreceptor afferent fibers in the caudal medulla (Cireillo, 1983), the nucleus tractus solitarii (NTS) participates in central cardiovascular control by functioning as an integration site for baroreceptive information. The major neurotransmitter that exerts an inhibitory modulation on the baroreceptor inputs in the NTS is gamma-aminobutyric acid (GABA) (Bennett et al., 1987; Kubo and Kihara, 1988; Yin and Sved, 1996; Zhang and Mifflin, 1998). Direct application of GABA agonist into the NTS inhibits the BRR response (Tsukamoto and Sved, 1993; Okada and Bunag, 1995; Zhang and Mifflin, 1998). Blockade of $GABA_A$ or $GABA_B$ receptors with their specific antagonists, on the other hand, decreases arterial pressure (Okada and Bunag, 1995; Durgam et al., 1999). GABAergic neurotransmission in the NTS also mediates the suppression of BRR response by activation of the forebrain structures (Feldman and Felder, 1991; Owens et al., 1999).

The mechanism of RVLM-induced suppression of cardiac BRR response has not been systemically inves-

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tigated. A recent report from our laboratory (Len and Chan, 1999) demonstrated that glutamatergic neurotransmission in the RVLM is involved in the suppression of reflex bradycardia induced by activation of the parabrachial nucleus in the dorsolateral pontine tegmentum. The presence of a direct GABAergic projection to the NTS from the RVLM has been reported (Loewy et al., 1981; Livingston and Berger, 1989). An immediate corollary, which forms our working hypothesis, is that GABAergic neurotransmission in the NTS participates in the suppression of reflex bradycardia elicited by glutamatergic activation of the RVLM. The present study was designed to assess this hypothesis. We measured changes evoked by glutamatergic activation of the RVLM in GABA concentration by in vivo microdialysis in the NTS and correlated them with RVLM-induced suppression of cardiac BRR response. Pharmacologic manipulations were used to identify the subtype(s) of GABA receptors in the NTS involved in the RVLM-induced BRR suppression. Our results suggest that GABA released in the NTS may participate in cardiac BRR suppression induced by glutamatergic activation of the RVLM, via an action on both $GABA_A$ and $GABA_B$ receptor subtypes.

MATERIALS AND METHODS

Animal preparation

Adult male Sprague-Dawley rats (230–250 g) obtained from the Experimental Animal Center of the National Science Council, Taiwan, were used. Animals were initially anesthetized with an intraperitoneal injection of pentobarbital sodium (40 mg/kg). Preparatory surgery included intubation of the trachea and cannulation of the right femoral artery and both femoral veins. Systemic arterial pressure (SAP) was monitored from the cannulated artery via a pressure transducer (Statham P23 ID) and a pressure processor amplifier (Gould 20-4615-52). Heart rate (HR) was determined by a cardiotachometer (Gould 20-4615-65) triggered by the arterial pressure pulses. Pulsatile and mean systemic arterial pressure (MSAP), as well as HR, were recorded simultaneously on a polygraph (Gould RS 3400). The head of the animal was thereafter placed in a stereotaxic headholder (Kopf 1404), with the rest of the body positioned on a heating pad and elevated to a suitable position for maintenance of a patent airway.

During the experiment, animals were artificially ventilated using a rodent respirator (Harvard 683) to maintain end-tidal CO_2 within 4–5%, as monitored by a capnograph (Datex Normocap). This was carried out to minimize confounding cardiovascular changes secondary to respiratory perturbations. Anesthetic maintenance was provided by intravenous infusion of pentobarbital sodium at 15–20 mg/kg/h. This management scheme was found to provide stable anesthesia while preserving the capability of cardiovascular regulation, including the BRR response (Yang et al., 1996). All data were collected from animals with a maintained rectal temperature of 37°C, with a steady MSAP above 90 mmHg throughout the experiment.

Fabrication and calibration of microdialysis probe

To achieve site specificity and enhance detection sensitivity, coaxial microdialysis probes with an active exchange area compatible with the RVLM or NTS were fabricated as modified from the procedures developed in our laboratory (Tsou et al., 1994a; Hwang et al., 1998). These microdialysis probes had an active exchange area of 350–400 μm in length and 220 μm in diameter. The membrane (Spectra/Por RC; 200-μm ID, 220-μm OD) was made of regenerated cellulose and had a molecular size cutoff of 13,000 Da. The inlet tubing (FEP-tubing, 1.2 μl/100 mm, CMA) together with outlet silica capillary tubing (Polymicro Technologies, Phoenix, AZ; 40-μm ID, 105-μm OD) of the coaxial probe were connected to a 23-gauge stainless steel cannula via PE-50 tubing. The outlet silica tubing, which protruded approximately 5.0 mm from the cannula, was fitted into the regenerated cellulose dialysis membrane to construct the head of the dialysis probe. One end of the dialysis membrane was securely sealed with polyimide resin (Alltech, Deerfield, IL) and its thickness was restricted to less than 100 μm (Alltech). The other end of the dialysis membrane was fitted into the stainless steel cannula. All junctions of the stainless steel cannula with the inlet, outlet, and dialysis membrane were securely anchored with epoxy glue. Under a high-power dissection microscope, the dialysis membrane was coated with polyimide resin. A resin-free length of 350–400 μm was left at the tip of the dialysis probe for active exchange area.

Fabricated microdialysis probes were calibrated at random for in vivo recovery rate. For this purpose, a probe was placed stereotaxically into the RVLM or NTS and known concentrations of L-glutamate (10, 50, or 100 μM) or GABA (0.5, 1.0, or 2.5 μM) were infused using a syringe microliter infusion pump (CMA/102, Carnegie Medicin) at a rate of 1 μl/min. In vivo recovery was calibrated based on procedures reported previously (Menacherry et al., 1992; Tsou et al., 1994a, b; Miele et al., 1996). The in vivo recovery rate of our microdialysis probes for L-glutamate in the RVLM and for GABA in the NTS, derived from the slope of the regression line, was respectively 21.8 ± 3.6% ($n = 5$) and 9.4 ± 0.6% ($n = 6$).

Sampling procedures and measurement of amino acid content

The microdialysis probe was lowered into the RVLM at 4.5–5.0 mm posterior to the lambda, 1.8–2.1 mm lateral to midline and 8.0–8.5 mm from the surface of

the cerebellum. The coordinates for the NTS were −4.5 to −5.0 mm from the interaural bars, 0.3 to 1.0 mm lateral to the midline, and 0.5 to 0.8 mm below the dorsal surface of the medulla. To minimize possible damage of the parasympathetic preganglionic neurons in nucleus ambiguus en route, the probe was lowered to the RVLM at 5–10° from the vertical axis. Brain tissue was continuously perfused at 1.0 μl/min during the period of stabilization with an artificial cerebrospinal (aCSF) solution (in mM: 149 NaCl, 2.8 KCl, 1.2 $CaCl_2$, 1.2 $MgCl_2$, 26 $NaHCO_3$, pH 7.4) by means of a microliter infusion pump (CMA/102, Carnegie Medicin). Collection of dialysate usually commenced 150–180 min after insertion of the dialysis probe. Samples were collected every 20 min and were stored in Eppendorf tubes kept on ice.

The concentration of L-glutamate or GABA in each sample was immediately quantified by high-performance liquid chromatography (HPLC) coupled with fluorescent detection with *o*-phthalaldehyde (OPA) derivatization, as modified from the method reported previously (Jones and Gilligan, 1983). The derivatization reagent was prepared by dissolving OPA (27 μg, Sigma, St. Louis, MO) in 1 ml absolute methanol. To this solution was added 5 μl of β-mercaptoethanol (Sigma) and then diluted up to 10 ml with 0.25 M boric acid (Sigma) buffer (pH 9.5). One volume (20 μl) of standard solution for amino acid or dialysate was mixed with two volumes (40 μl) of the derivatization reagent solution. The contents were mixed for 90 sec at 6°C and injected into HPLC system using an automatic sample injector (CMA 200, Carnegie Medicin).

The HPLC system consisted of a dual-piston syringe pump (PM80, Bioanalytical System) and a fluorescence detector (FL-45A, Bioanalytical System). The derivatized amino acids were detected at an excitation wavelength of 340 nm and an emission wavelength of 450 nm. The mobile phase, comprised of 0.1 M potassium acetate buffer solution (pH 5.5; Merck, Darmstadt, Germany) and 100% methanol (LC grade, Merck), was pumped at 1.0 ml/min through a reverse-phase C-18 column (3 μm, 150 × 4.6 mm, Alltech). The column temperature was maintained at 40°C with temperature controller (Timberline TL-50) and column heater (Timberline TL-30). The gradient elusion program was run from a 20–60% linear gradient of methanol for 7 min, followed by isocratic gradient at 60% for 1 min. The mobile phase was followed by a 4-min washing step (90% methanol) and the column was equilibrated with 20% methanol. Peak area analysis of chromatograms was performed using a computer-based system (Chem. Lab Data Station, SISC) and subsequently converted to concentrations in picomoles based on standard amino acid solutions (1.0×10^{-9} to 1.0×10^{-6} M). The minimal detection limit of measurement for L-glutamate or GABA was at the range of 50 fmol for L-glutamate and 20 fmol for GABA. This detection limit was comparable to previous studies (Kihara et al., 1989; Morari et al., 1993; Tanganelli et al., 1994; Kehr, 1998; Kodama et al., 1998) that used the same method. The concentration of amino acid was normalized to a percentage of pretreatment control to compensate for variations between animals and allow for comparison between treatment groups.

Evaluation of cardiac baroreceptor reflex response

As in our previous studies (Chan et al., 1998; Hwang et al., 1998; Len and Chan, 1999), the sensitivity of BRR control of HR was evaluated by measuring the reflex bradycardia in response to transient hypertension evoked by an i.v. bolus administration of phenylephrine (5 μg/kg, 250 μl). The quotient calculated from the peak reflex decrease in HR for a given peak increase in MSAP (beats/min/mmHg) was used as the index for cardiac BRR response. The control value of this quotient, taken from all the experimental groups, amounted to 1.04 ± 0.07 (mean ± SEM, $n = 70$). In each treatment group, this quotient was further normalized to a percentage of pretreatment control to compensate for variations between animals and to allow for comparison between treatment groups.

Microinfusion of L-glutamate into the Rostral Ventrolateral Medulla and Estimation of Extracellular Concentration of L-glutamate

In some experiments, L-glutamate was microinfused into the RVLM via the microdialysis probe based on the principle of reverse microdialysis (Chan et al., 1995; Chan and Chan, 1999). The extracellular concentration of L-glutamate in the RVLM was estimated according to the equations reported previously (Chan and Chan, 1999; Tsou et al., 1994a, b). In essence, since L-glutamate was being microinfused continuously into the RVLM, its concentration in this medullary site may be estimated according to the following equation:

$$([\text{Glutamate}]_{\text{RVLM}})_n = \sum_{i=1}^{n} (([\text{Glutamate}]_{\text{IN}} - [\text{Glutamate}]_{\text{OUT}})_i - ([\text{Glutamate}]_{\text{IN}} - [\text{Glutamate}]_{\text{OUT}})_{SS}) + ([\text{Glutamate}]_B)$$

where n is the succession of sampling, $i = 1$ to n; $[\text{Glutamate}]_{\text{RVLM}}$ is the estimated extracellular concentration of L-glutamate in the RVLM; $[\text{Glutamate}]_{\text{IN}}$ is the concentration of L-glutamate in the dialysate collected from the RVLM; $([\text{Glutamate}]_{\text{IN}} - [\text{Glutamate}]_{\text{OUT}})_{SS}$ is the steady-state condition when the change in the difference between concentrations of L-glutamate in the perfusate and dialysate among successive samples is within 10%;

and $[\text{Glutamate}]_B$ is the mean baseline concentration of L-glutamate in the RVLM.

The microdialysis probe simply served as a sampling device when aCSF replaced L-glutamate as the perfusate. The estimated concentration of L-glutamate in the RVLM can be estimated by the equation:

$$[\text{Glutamate}]_{\text{RVLM}} = [\text{Glutamate}]_{\text{OUT}}/\text{probe in vivo recovery rate}$$

where $[\text{Glutamate}]_{\text{RVLM}}$ is the estimated extracellular concentration of L-glutamate in the RVLM; and $[\text{Glutamate}]_{\text{OUT}}$ is the concentration of L-glutamate in the dialysate collected from the RVLM.

Microinjection of chemical agents

Microinjection bilaterally of chemicals into the RVLM or NTS was executed with a glass micropipette connected to a 0.5-μl microsyringe. The stereotaxic coordinates used were the same as in microdialysis. A total volume of 50 nl was delivered into each side over 1–2 min to allow for complete diffusion of the chemical agent. Injection of the same amount of aCSF served as control for possible volume effect of microinjection. All microinjection solutions contained 1% Evans Blue to aid in histologic verification of the injection site.

To investigate the participation of GABAergic neurotransmission at the NTS in the modulation of BRR response by activation of RVLM by L-glutamate (1 nmol), a $GABA_A$ receptor antagonist, bicuculline methiodide (1 or 5 pmol; Sigma) or a $GABA_B$ receptor antagonist, 2-hydroxy-saclofen (100 or 500 pmol; RBI, Natick, MA), was microinjected into the bilateral NTS. The effect of each chemical treatment on basal BRR response or on modulation of BRR response induced by microinjection of L-glutamate into the bilateral RVLM was evaluated for 60 min postinjection. The dose of bicuculline methiodide or 2-hydroxy-saclofen was adopted from studies (Avanzino et al., 1994; Okada and Bunag, 1995; Suzuki et al., 1999) in which they were used for the same purpose as in our experiments.

All solutions were aliquotted, stored frozen, and thawed immediately before use during the experiment. Phenylephrine was freshly prepared with 0.9% saline.

Histology

The brain was removed at the conclusion of each experiment and fixed in 30% sucrose in 10% formaldehyde-saline solution for at least 2 days. The position of the microdialysis probe or the tip of the microinjection needle in the RVLM or NTS was histologically verified on 25-μm frozen sections stained with 1% neutral red.

Statistical analysis

Data are presented as mean ± SEM. The temporal effect of experimental treatments was statistically assessed using two-way analysis of variance (ANOVA) with repeated measures. This was followed by the Scheffé or Dunnett multiple-range test for a posteriori comparison of means at comparable time intervals. Difference was considered statistically significant at $P < 0.05$.

RESULTS

Temporal changes in extracellular concentration of L-glutamate in the RVLM, GABA concentration in the NTS, and cardiac BRR response

A previous study from our laboratory demonstrated that activation of PBN resulted in suppression of BRR response via glutamatergic neurotransmission in the RVLM (Len and Chan, 1999). To establish a relationship between an increase in extracellular L-glutamate concentration in the RVLM, change in GABA concentration in the NTS, and inhibition of cardiac BRR response, L-glutamate (50 μM) was infused into the RVLM via the microdialysis probe based on the principle of reverse microdialysis (Chan et al., 1995; Chan and Chan, 1999).

Direct microinfusion of L-glutamate (50 μM, $n = 7$) unilaterally into the RVLM evoked an increase in the estimated extracellular concentration of L-glutamate in this medullary nucleus (Fig. 1A). Such an increase followed a time course that reached it peak at 60–80 min postinfusion and underwent a rapid decrease upon replacing L-glutamate with aCSF as the perfusate. The estimated extracellular concentration of L-glutamate at 60–80 min after infusion of this excitatory amino acid into the RVLM was 11.30 ± 0.78 μM ($n = 7$). Histologic verification confirmed that the tip of the microdialysis probe was located within the confines of the RVLM (Fig. 2A).

Microinfusion of L-glutamate unilaterally into the RVLM also elicited a significant inhibition of the cardiac BRR response (Fig. 1B) that paralleled the temporal elevation in the estimated extracellular concentration of L-glutamate in the RVLM. Interestingly, we also found a temporal increase of GABA concentration in the dialysate collected from the ipsilateral NTS that paralleled the time-course of increase in estimated extracellular concentration of L-glutamate in the RVLM (Fig. 1C). At 60–80 min after infusion of L-glutamate into the RVLM, the dialysate level of GABA in the NTS increase from a basal level of 14.2 ± 1.1 nM (mean ± SEM, $n = 7$) to 27.6 ± 3.0 nM ($n = 7$). Histologic verification also confirmed that the tip of the microdialysis probes was located within the NTS (Fig. 2B).

We confirmed that the inhibition of reflex bradycardia by microinfusion of L-glutamate into the RVLM was not due to alterations in the transiently evoked hypertension. Phenylephrine elicited similar degrees of increase in MSAP before and at 80 min after L-glutamate

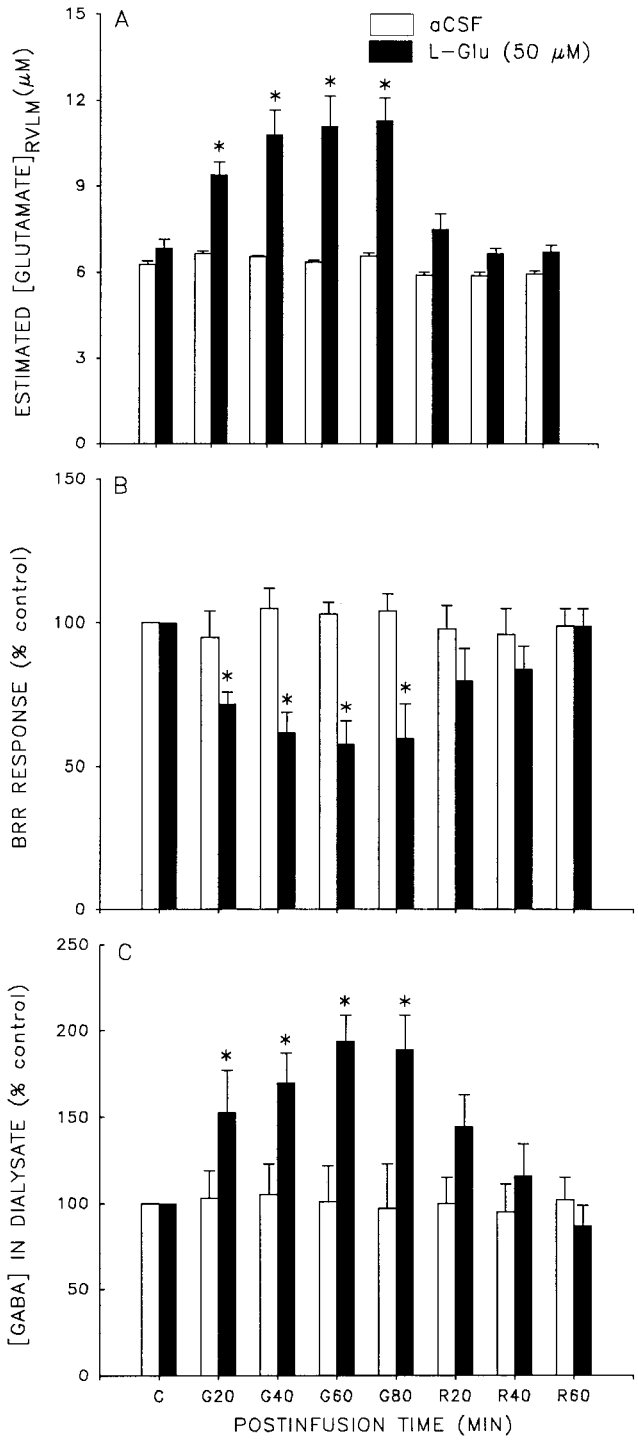

Fig. 1. Time course of simultaneous alterations in estimated extracellular L-glutamate concentration ($[GLUTAMATE]_{RVLM}$) in the area containing rostral ventrolateral medulla (**A**), cardiac baroreceptor reflex (BRR) response (**B**), and GABA levels ([GABA]) in the dialysate collected from ipsilateral nucleus tractus solitarius (**C**) before (C), during (G20 to G80), and after (R20 to R60) microinfusion unilaterally into the RVLM of aCSF ($n = 6$) or L-glutamate (L-Glu, 50 μM; $n = 7$) at 1 μl/min. Values are mean ± SEM. *$P < 0.05$ vs. aCSF group in the Scheffé multiple-range test.

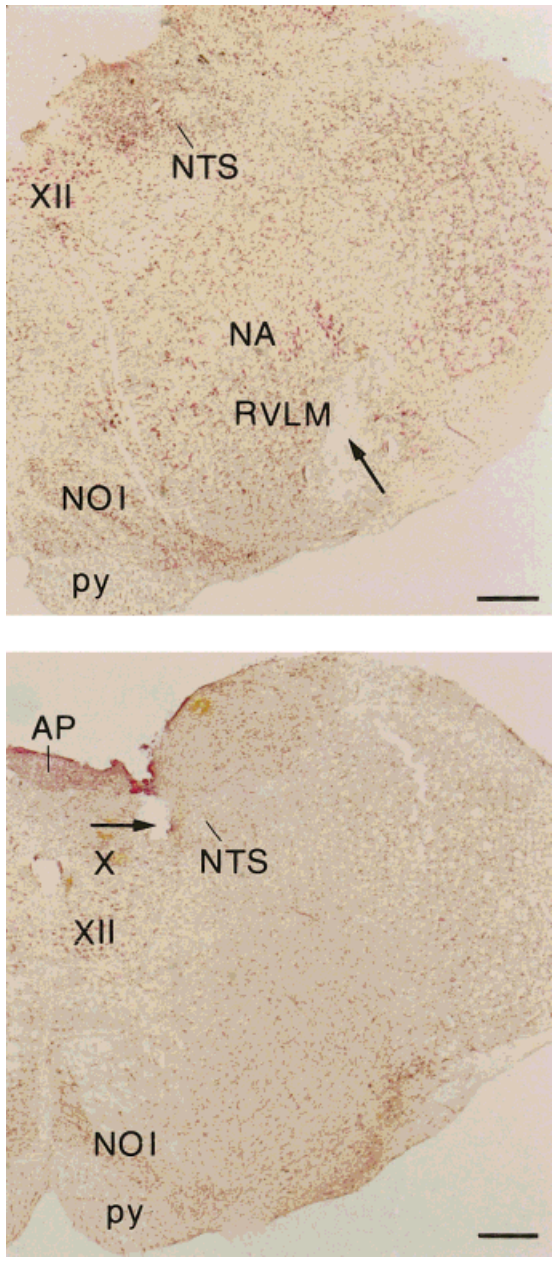

Fig. 2. Representative photomicrographs showing location of the tip of microdialysis probes in the area containing RVLM (**A**) or NTS (**B**). AP, area postrema; NA, nucleus ambiguus; NOI, nucleus olivaris inferior; NTS, nucleus tractus solitarius; RVLM, rostral ventrolateral medulla; V, nucleus and tractus trigemini spinalis; X, nucleus dorsalis nervi vagi; XII, nucleus nervi hypoglossi; py, tractus pyramidalis. Scale bar = 500 μm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

infusion (+ 46.3 ± 4.8 vs. + 48.2 ± 5.4 mmHg, $n = 13$).

Figure 3 depicts the site specificity of the increase in GABA concentration in the dialysate of NTS and suppression of cardiac BRR response to microinfusion of L-glutamate into the RVLM. We found that, at the same infusion rate, application of L-glutamate (50 μM, $n = 6$) (Fig. 3A) into areas adjacent to the RVLM, e.g.,

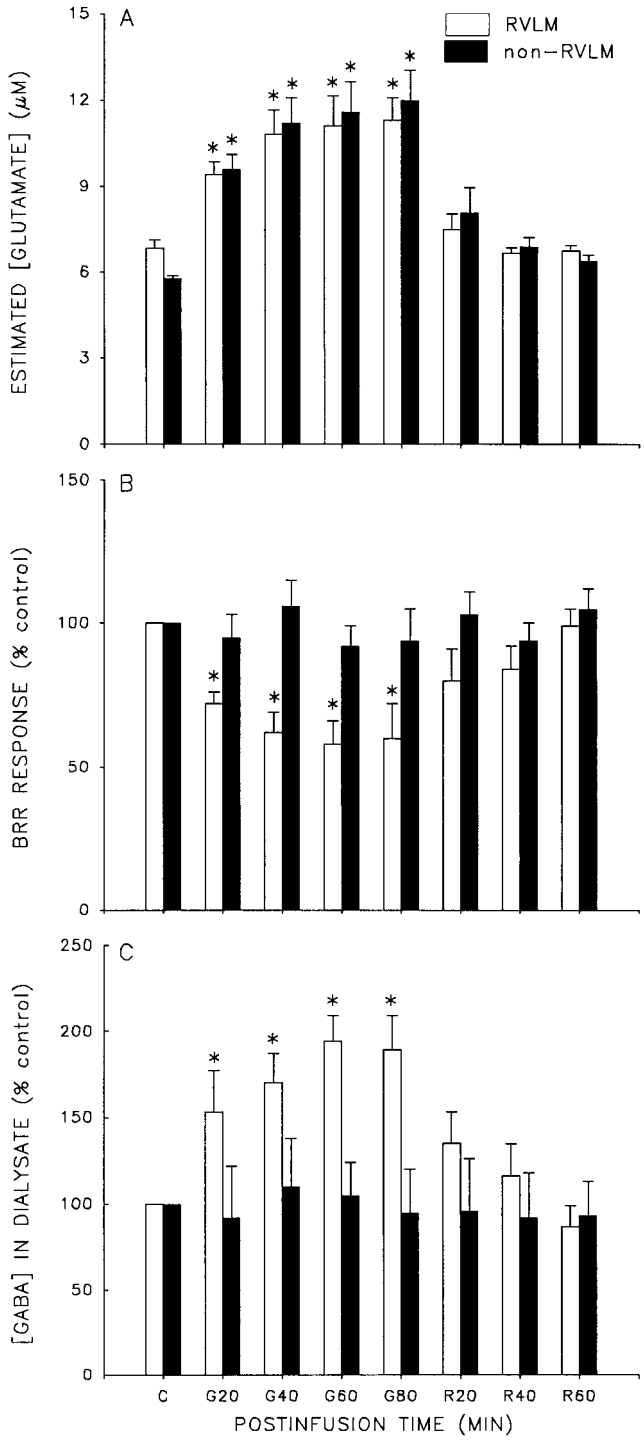

Fig. 3. Time course of simultaneous alterations in estimated extracellular L-glutamate concentration ([GLUTAMATE]) (**A**), cardiac baroreceptor reflex (BRR) response (**B**), and GABA levels ([GABA]) in the dialysate collected from ipsilateral nucleus tractus solitarius (**C**) before (C), during (G20 to G80), and after (R20 to R60) microinfusion unilaterally into the spinal trigeminal nucleus or paragignatocellular reticular nucleus (non-RVLM) of L-glutamate (L-Glu, 50 μM; $n = 6$) at 1 μl/min. Data from Fig. 1 on microinfusion of L-Glu into the area containing rostral ventrolateral medulla (RVLM) are reproduced for comparison. Values are mean ± SEM. *$P < 0.05$ vs. baseline control in the Dunnett multiple-range test.

spinal trigeminal nucleus or paragigantocellular reticular nucleus, resulted in a temporal change in the estimated extracellular concentration of L-glutamate that was comparable to that depicted in Figure 1A. Nonetheless, these changes in L-glutamate concentration were not accompanied by concomitant alterations in cardiac BRR response (Fig. 3B) or extracellular concentration of GABA in the ipsilateral NTS (Fig. 3C).

Involvement of GABA receptor subtypes at the NTS in RVLM-induced cardiac BRR suppression

We recognize that a correlated increase in extracellular concentration of L-glutamate in the RVLM, elevation in GABA level in the NTS, and inhibition of cardiac BRR response do not confer causation. The existence of such a causative link, and the GABA receptor subtypes in the NTS that are engaged in the suppression of cardiac BRR response evoked by L-glutamate in the RVLM, were subsequently delineated using GABA antagonists. Local application of bicuculline methiodide (1 or 5 pmol, $n = 7$) or 2-hydroxy-saclofen (100 or 500 pmol, $n = 6$) into the bilateral NTS significantly reversed cardiac BRR suppression induced by microinjection of L-glutamate (1 nmol) into the bilateral RVLM (Fig. 4). Concomitant coadministration of bicuculline methiodide and 2-hydroxy-saclofen (1 pmol + 100 pmol, $n = 6$), however, did not promote further blockade of the RVLM-induced suppression of cardiac BRR response (Fig. 4). At the higher doses used, both GABA antagonists exerted a mild but significant increase in basal cardiac BRR response. We also noted that administration of bicuculline methiodide, but not 2-hydroxy-saclofen, into the bilateral NTS promoted a dose-dependent decrease in baseline MSAP and HR (Table I).

DISCUSSION

Excitatory amino acids, in particular glutamate, has been demonstrated to be a major excitatory neurotransmitter in the RVLM involved in the regulation of cardiovascular functions. Exogenous application of L-glutamate into the RVLM increases SAP (Ross et al., 1984; Kubo et al., 1989). Blockade of endogenously released glutamate at the RVLM by NMDA or non-NMDA receptors antagonizes pressor response during muscle contraction (Kobayashi et al., 1997). This excitatory amino acid in the RVLM has also been demonstrated to mediate the suppression of BRR response by angiotensin II (Moriguchi et al., 1995) and by activation of pontine and forebrain structures (Len and Chan, 1999; Owens et al., 1999). Moreover, both NMDA and non-NMDA receptors in the RVLM are involved in the suppression of reflex bradycardia promoted by glutamate (Len and Chan, 1999). Pathophysiologically, elevated glutamate level has been reported

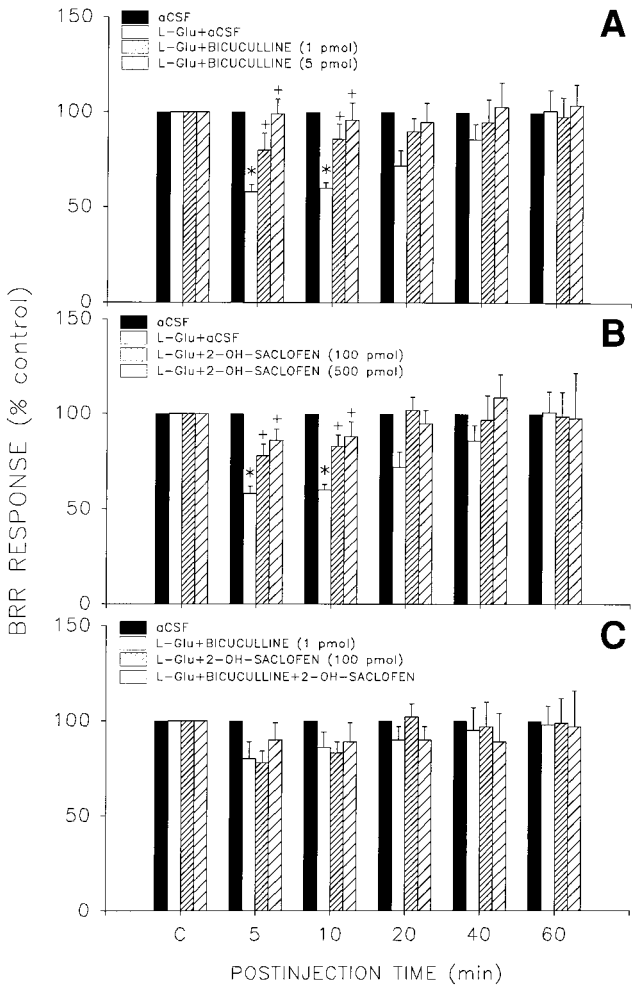

Fig. 4. Temporal effect of microinjection into the bilateral NTS of aCSF, bicuculline methiodide (1 or 5 pmol, **A**), 2-hydroxy-saclofen (100 or 500 pmol, **B**) or bicuculline methiodide + 2-hydroxy-saclofen (1 p mol + 100 pmol, **C**) on the suppression of cardiac BRR response induced by microinjection into the bilateral RVLM of L-Glu (1 nmol). Values are mean ± SEM, $n = 6–7$ animals per groups. $^{*}P < 0.05$ vs. aCSF group, and $^{\dagger}P < 0.05$ vs. L-GLU + aCSF group in the Scheffé multiple-range test.

in the RVLM of spontaneously hypertensive rats (SHR) (Kubo et al., 1989). An augmented responsiveness to glutamate in the RVLM may be responsible for the reduced BRR sensitivity and elevated SAP seen in the SHR (Tsuchihashi et al., 1998). On the basis of combined results from physiologic, neurochemical, and pharmacologic experiments, a major contribution of the present study is the demonstration that GABAergic neurotransmission in the NTS may participate in the suppression of BRR control of HR on glutamatergic activation of the RVLM. Of note is that at its peak the estimated extracellular concentration of L-glutamate on microinfusion of the excitatory amino acid into the RVLM amounted to 11.30 ± 0.78 μM. This is comparable to the extracellular concentration of L-glutamate (10–15 μM) evoked in the RVLM by an electrical stimulation of PBN that also induces cardiac BRR inhibition (unpublished data).

Whereas a direct GABAergic projection to the NTS from the RVLM has been demonstrated (Loewy et al., 1981; Livingston and Berger, 1989), the physiologic relevance of the GABA released in the NTS on activation of the RVLM has hereto not been discerned. Thus, results of the present study are of interest since they provide direct evidence that linked the evoked release of GABA in the NTS with the suppression of cardiac BRR response elicited by glutamatergic activation of the RVLM. By monitoring the simultaneous fluctuations in GABA concentration in the dialysate collected from the NTS and suppression of cardiac BRR response, we found a high correlation between temporal changes of these two events in response to RVLM activation by L-glutamate. These findings, at the same time, substantiate the functional significance of the RVLM-NTS GABAergic pathway in baroreflex control of blood pressure.

Despite a wealth of studies that report changes in extracellular GABA concentration in the forebrain structures (Morari et al., 1993; Tanganelli et al., 1994; Giggs et al., 1995; Lindefors et al., 1997; Smolders et al., 1997), information on GABA concentration in the NTS detected by in vivo microdialysis is not available. As such, another major contribution of the present study is to provide information on basal and evoked levels of GABA in this medullary nucleus. The basal level of GABA that we measured in the dialysate collected from the NTS was in agreement with that collected from nucleus accumbens (Tanganelli et al., 1994), dorsolateral neostriatum (Morati et al., 1993), and medial prefrontal cortex (Lindefors et al., 1997) in anesthetized rats. On the other hand, it is lower than that collected from substantia nigra of freely moving rats (Biggs et al., 1995; Smolders et al., 1997). Such differences may be a result of the state of consciousness as well as the abundance of GABAergic terminals and interneurons in the substantia nigra.

It has been debated in the literature as to whether extracellular GABA measured by in vivo microdialysis reflects the level of this amino acid released from the neuronal exocytotic pool (Timmerman and Westerink, 1997). In support of the vesicular origin of glutamate in dialysate, basal or evoked levels of glutamate have been reported to be decreased in the presence of the fast sodium-channel blocker tetrodotoxin (TTX) (Tanganelli et al., 1994; Grobin and Deutch, 1998), or on infusion with Ca^{2+}-free aCSF (Biggs et al., 1995; Kolachana et al., 1997). On the other hand, a lack of blunting effect by TTX or calcium channel blocker on basal or evoked levels of glutamate in the brain has also been shown (Hondo et al., 1995; Timmerman and Westerink, 1997). In this regard, preliminary results demonstrated that perfusion of Ca^{2+}-free aCSF presented no discernable effect on the basal level of GABA

TABLE I. Effect of microinjection into the bilateral NTS of $GABA_A$ receptor antagonist, bicuculline methiodide (1 or 5 pmol), or $GABA_B$ receptor antagonist, 2-hydroxy-saclofen (100 or 500 pmol) on mean systemic arterial pressure (MSAP), heart rate (HR), and cardiac BRR response

	MSAP (mmHg)		HR (bpm)		BRR (bpm/mmHg)	
	Basal	Change after treatment	Basal	Change after treatment	Basal	Change after treatment
aCSF	110 ± 4	−2 ± 1	385 ± 9	−4 ± 2	0.98 ± 0.06	+0.04 ± 0.02
Bicuculline						
1 (pmol)	114 ± 5	−15 ± 3*	378 ± 6	−23 ± 9*	1.01 ± 0.07	+0.05 ± 0.04
5 (pmol)	110 ± 3	−20 ± 6*	381 ± 9	−28 ± 7*	0.97 ± 0.04	+0.17 ± 0.03*
Saclofen						
100 (pmol)	108 ± 4	−4 ± 6	380 ± 8	−5 ± 8	1.03 ± 0.08	+0.09 ± 0.05
500 (pmol)	104 ± 6	−9 ± 5	376 ± 9	−14 ± 9	0.99 ± 0.06	+0.22 ± 0.06*

NTS, nucleus tractus solitarii; aCSF, artificial cerebrospinal fluid. Values are presented as mean ± SEM, $n = 6–7$ animals per group. *$P < 0.05$ as compared with aCSF in Scheffé multiple-range test.

(14.9 ± 1.5 nM, $n = 3$ vs. 14.2 ± 1.1 nM, $n = 7$) in the dialysate collected from the NTS. However, the increase in GABA level in the ipsilateral NTS (20.7 ± 2.4 nM, $n = 3$ vs. 27.6 ± 3.0 nM, $n = 7$) on microinfusion of L-glutamate (50 μM) into the unilateral RVLM was attenuated. Together with our demonstration of site specificity in the increase in extracellular GABA levels in the NTS (cf. Fig. 3), it is most likely that activation of the RVLM-NTS GABAergic projection and the resultant neuronal exocytotic release may account for this increase. Whether the measured increases may also originate from non-neuronal pools (e.g., glial cells), however, remains to be delineated.

Our results with GABA antagonists provide the crucial link between glutamatergic activation at the RVLM, increase in GABA in the NTS, and inhibition of cardiac BRR response. At the receptor level, they further demonstrate that both $GABA_A$ and $GABA_B$ receptors in the NTS are involved in the process. Since concomitant application of bicuculline methiodide and 2-hydroxy-saclofen did not further antagonize the RVLM-induced BRR suppression, we speculate that there may be an interaction between $GABA_A$ and $GABA_B$ receptors at the NTS in the inhibition of BRR response. Interactions of $GABA_A$ and $GABA_B$ receptors have been demonstrated in the NTS to modulate the BRR response (Sved and Tsukamoto, 1992). We are also aware that there is an abundance of intrinsic GABA-containing interneurons in the brain stem nuclei that subserve autonomic control of blood pressure (Meeley et al., 1985). Thus, apart from the direct GABAergic projection from the RVLM to the NTS, the engagement indirectly of GABA interneurons in the NTS in the inhibitory modulation of cardiac BRR response by the RVLM cannot be ruled out.

Consistent with previous reports (Sved and Tsukamoto, 1992; Yin and Sved, 1996; Durgam et al., 1999), we found a mild but significant enhancement in the basal BRR response to microinjection into the bilateral NTS of the higher dose of $GABA_A$ or $GABA_B$ receptor antagonist. This implies a tonic modulatory role for GABA at the NTS in BRR control of HR. It also suggests that the blunting of RVLM-induced suppression of cardiac BRR response by either GABA receptor antagonist may simply result from cancellation of two physiologic responses. This possibility, however, is unlikely, since microinjection into the bilateral NTS of the low dose of bicuculline methiodide and 2-hydroxy-saclofen similarly antagonized the RVLM-induced BRR suppression while producing no discernible effect on basal BRR response.

In conclusion, on the basis of in vivo microdialysis in conjunction with pharmacologic treatment, the present study demonstrates that GABA released in the NTS functions as a chemical mediator in the RVLM-induced suppression of cardiac BRR response by acting on both $GABA_A$ and $GABA_B$ receptors in the NTS. Neurons in the RVLM play an important role in central cardiovascular regulation. In addition to the well-established role of tonically maintaining the excitability of sympathetic preganglionic neurons in the spinal cord (Guyenet, 1990; Reis et al., 1994), this medullary nucleus participates in BRR control of HR by modulating the excitatory inputs from the NTS to the parasympathetic preganglionic neurons in the dorsal motor nucleus of the vagus (DMV) and nucleus ambiguus (NA) (Wang and Li, 1988). On the other hand, microiontophoretic application of GABA inhibits the excitability of the barosensitive neurons in the NTS (Feldman and Felder, 1991; Suzuki et al., 1993). Taken together with the present results, it is conceivable that GABA released in the NTS upon glutamatergic activation of the RVLM may reduce the excitatory inputs from the NTS to DMV and/or NA, resulting in an inhibition of reflex bradycardia in response to baroreceptor activation.

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