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Nitric Oxide (NO) Inhibites the Neuronal Activities in the Rat Nucleus Tractus Solitarius

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Nitric oxide (NO) system has been implicated in a wide range of physiological functions in the nervous system. However, the role of NO in regulating the neural activity in the gustatory zone of nucleus tractus solitarius (NTS) has not been established. The present study was aimed to investigate the role of NO in the gustatory NTS neurons. Sprague-Dawley rats, weighing about 50 g, were used. Whole cell patch recording and immunohistochemistry were done to determine the electrophysiological characteristics of the rostral gustatory nucleus of the tractus solitarius and distribution of NO synthases (NOS). Neuronal NOS (nNOS) immunoreactivity was strongly detected along the solitary tract extending from rostral to caudal medulla. Resting membrane potentials of NTS neurons were −49.2±2 mV and action potential amplitudes were 68.5±2 mV with a mean duration measured at half amplitude of 1.7±0.3 ms. Input resistance, determined from the response to a 150 ms, −100 pA hyperpolarizing current pulse, was 385±15 MΩ. Superfusion of SNAP or SNP, NO donors, produced either hyperpolarization (68%), depolarization (5%), or no effect (27%). The hyperpolarization was mostly accompanied by a decrease in input resistance. The hyperpolarization caused by SNAP or SNP increased the time to initiate the first action potential, and decreased the number of action potentials elicited by current injection. SNP or SNAP also markedly decreased the number of firing neural discharges of the spontaneous NTS neural activity under zero current. Superfusion of L-NAME, a NOS inhibitor, slightly depolarized the membrane potential and increased the firing rate of NTS neurons induced by current injection. ODQ, a soluble guanylate cyclase inhibitor, ameliorated the SNAP-induced changes in membrane potential, input resistance and firing rates. 8-Br-cGMP, a non-degradable cell-permeable cGMP, hyperpolarized the membrane potential and decreased the number of action potentials. It is suggested that NO in the gustatory NTS has an inhibitory role on the neural activity of NTS through activating soluble guanylate cyclase.

Key Words: Nitric oxide, Nucleus tractus solitarius

INTRODUCTION

Central gustatory system consists of nucleus tractus solitarius, thalamus, parabrachial nucleus and primary taste sensory cortex. The rostral extent of the nucleus of the solitary tract (rNTS) is the site of termination of afferent gustatory fibers (Hamilton & Norgren, 1984; Bradley et al, 1985). The neurons in rNTS receive gustatory and somatosensory afferent inputs from the chorda tympani, glossopharyngeal and superior laryngeal nerves (Hamilton & Norgren, 1984). Morphological analysis of the gustatory zone of the rat rNTS shows that it contains at least three distinct neuronal types (Lasiter et al, 1988). Fusiform neurons are characterized by an oval elongated soma and two large primary dendrites that exit the cell body at opposite poles. A third smaller dendrite may also be present in fusiform cells. Multipolar neurons have a stellate-shaped soma and three to five primary dendrites. Ovoid neurons have small soma and two or three primary dendrites that are generally thin, and sparsely and randomly branched. The small ovoid neurons are proposed to be interneurons in the medulla, whereas the multipolar and fusiform neurons project rostrally to the parabrachial nucleus and caudal NTS (Whitehead, 1990). Multipolar types are reported to be the predominant rostral projection neurons in the rat (Lasiter, 1991).

The neurotransmitters or neuromodulators detected localized immunohistochemically in the NTS include amino acids (GABA and glutamate), acetylcholine, amines (dopamine, norepinephrine, epinephrine, serotonin, histamine), and peptides (substance P, enkephalin, endorphin, calcitonin gene-related peptide, neuropeptide, angiotensin II,

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ABBREVIATIONS: NTS, nucleus tractus solitarius; CNS, central nervous system; nNOS, neuronal nitric oxide synthase; rNTS, rostral NTS; SNP, sodium nitroprusside; SNAP, S-nitroso-N-acetylpenicillamine; L-NAME, N^{ω}-nitro-L-arginine methyl ester.

vasoactive intestinal polypeptide, vasopressin, and atrial natriuretic peptide) (Maley, 1996). However, the role of these transmitters to relay gustatory information from taste buds to second order neurons in the rNTS has not been well identified.

Recently, it has been suggested that nitric oxide (NO), a small gaseous molecule, functions as intercellular signalling messengers in the brain (Dawson and Snyder, 1994). NO was firstly reported to mediate the N-metyl-D-aspartate (NMDA)-induced increase in cGMP in the central nervous system (CNS) (Garthwaite et al, 1988). Subsequently, it has been known to be implicated in a wide range of physiological roles as a neurotransmitter mediating non-adrenergic, non-cholinergic transmission, synaptic plasticity and long-term potentiation related to the learning and memory (Meller & Gebhart, 1993).

In neurons, NO is derived from L-arginine by a Ca^{2+}/calmodulin-activated NO synthase (NOS) (Knowles & Moncada, 1994). This enzyme is distributed widely throughout the brain (Bredt et al, 1991). The NOS-containing neurons exist in the rabbit central NTS and act as interneurons in a central pathway connecting esophageal afferents and efferents (Gai et al, 1995),indicating that the gustatory neurons in the rNTS may have NO system to play a physiological role in regulating the neuronal activity. However, existence of NOS in the gustatory neurons in the rNTS and the role of NO on regulation of the neuronal activity in the gustatory zone of the NTS have been not explored.

The present study was aimed to investigate the distribution of NOS in the rNTS and the action mechanism of NO in taste signaling in the rNTS, using immunohistochemistry and patch clamp techniques.

METHODS

NOS immunohistochemistry

Sprague-Dawley rats (weighing ~50 g) were anesthetized with pentobarbital sodium (I.P., 50 mg/kg) and perfused through the abdominal aorta with a solution of 4% paraformaldehyde in PBS containing (in mM): NaCl 137, KCl 2.7, Na_2HPO_4 4.3, and KH_2PO_4 1.4. Brain stems were removed and post-fixed in the same fixative solution for 2 h at 4°C and subsequently rinsed in PBS, cryoprotected with 30% sucrose in PBS for 58 h. Fifty μm horizontal sections were cut on a freezing microtome. Immunohistochemical staining was carried out using Vectastain Elite avidin-biocitin-peroxidase, with diaminobenzidine as a chromagen (Vector Laboratories, Burlingame, CA, USA). Purified monoclonal mouse anti-neuronal NOS (Transduction Laboratories, Lexington, KY, USA) was used as a primary antibody. Endogenous peroxidase was blocked by incubation in 3% H_2O_2 in water for 5 min. They were then incubated in 10% horse serum for 10 min to block non-specific reactions, and reacted in primary antibody diluted to 1 : 400 with 0.3% bovine serum albumin in PBS overnight. The tissues were washed with PBS and incubated in biotinylated anti-mouse IgG secondary antibody for 10 min. The sections were incubated in streptavidin/peroxidase complex for 5 min and developed with 3-amino-9-ethylcarbazole for 6 min. The sections were counterstained with Mayer's hematoxylin and mounted with Canada balsam for photography under light microscope.

Brain slice preparation

Brain slices containing the rostral 1.6 mm of the NTS were obtained from rats, as previously described by other investigator (King et al, 1993). Briefly, rats anesthetized with sodium pentobarbital (50 mg/kg) were decapitated and the brainstem were quickly removed, cooled and cut on a vibratome into horizontal slices of 300 μm thickness. The slices were incubated for at least 1 h in physiological salt solution (PSS) at room temperature. PSS contained (in mM): NaCl 124, KCl 2.5, $CaCl_2$ 2.4, $MgSO_4$ 0.65, $NaHCO_3$ 26, NaH_2PO_4 0.98, dextrose 10. The solution was gassed with 95% O_2 and 5% CO_2 to maintain the pH at 7.4. A slice was transferred to a recording chamber with a wide-mouthed pipette. During recordings, slices were secured in a chamber with nylon mesh and continuously superfused with oxygenated saline flowing at 1.5~2 ml/min.

Electrophysiological techniques

Unpolished patch electrodes, pulled 1.5 mm o.d. borosilicate filament glass (WPI, TW 150F-4) in two stages on a Narishige PP83 electrode puller, had tip resistances of 5~8 MΩ (bubble numbers 5.8~6.2). They were filled with a solution containing (in mM): potassium gluconate 130, HEPES 10, EGTA 10, $MgCl_2$ 1, $CaCl_2$ 1, ATP 2, and the pH was adjusted to 7.4 with KOH. The electrodes were positioned over the rNTS, which was easily identified when transluminated from below (Bradley & Sweazey, 1992). With the use of a hydraulic drive micromanupulator (Narighige), the electrodes were advanced into the rNTS and whole-cell recordings were made using the technique of Blanton et al (1989). Once a giga-ohm seal was formed and the patch ruptured, current stimulation protocols were performed and voltage data were acquired using the pCLAMP program (Axon Instruments). Current was injected into neurons using the bridge circuit of an Axoclamp 2B amplifier in current clamp mode. The indifferent electrode was Ag-AgCl wire connected to the extracellular solution. Neurons were separated into groups on the basis of their repetitive firing pattern induced by a 1,200 ms, 100 pA depolarizing pulse, preceded by a −100 pA hyperpolarizing pulse of increasing duration (0 and 50 ms). Throughout the experiment, membrane input resistance was measured using 150 ms, −100 pA hyperpolarizing current pulses at 0.15 Hz. These pulses caused a change in membrane potential along the linear phase of the current-voltage relationship and input resistance was measured at saturation. The average length of a recording period was 35 min.

Previous studies have shown that neurons in rNTS can be divided into 4 groups on the basis of their repetitive discharge patterns (Bradely & Sweazey, 1992; King et al, 1993). These 4 groups of neurons were also recognized in the present study, and group I, II or III neurons were used.

Data analysis

The Clamfit program (Axon Instrument) was used to analyze biophysical properties of neurons in response to various drugs. Neurons studied demonstrated a resting membrane potential to be more negative than −40 mV, action potential with overshoot, and an input resistance 100 mΩ (determined from the response to a 150 ms, −100 pA, hyperpolarizing current pulse).

A response to a drug was defined as a noticeable change in membrane potential (>1 mV), which usually returned to baseline after washout. The magnitude of responses was determined by comparing the membrane potential immediately prior to the application of drug to that during the peak of the response. In addition, action potential number, initial time and duration (measured at the point of half amplitude) were compared. The input resistances before and during application of drugs were compared by analyzing the responses to a −100 pA, 150 ms hyperpolarizing current pulse applied at appropriate times.

Pharmacology

By a system of valves, PSS containing various drugs was superfused over the slice for 5~8 min, and took 2 min to arrive at the slice chamber. Control PSS was superfused over the slice for at least 4 min, before switching to another drug. 1H-[1,2,4] oxadiazolo [4,3-a] quinoxaline-1-one (ODQ) and S-nitroso-N-acetyl-penicillamine (SNAP) were prepared in DMSO. Final DMSO concentration never exceeded 0.01%. Sodium nitroprusside (SNP), N^{ω}-nitro-L-arginine methyl ester (L-NAME) and 8-Bromo-cGMP (8-Br-cGMP) were prepared in normal PSS. All drugs used were obtained from Sigma chemical Co. except ODQ and SNAP, which were supplied by Research Biochemicals Incorporated.

RESULTS

Localization of nNOS in NTS

NTS was cylindrically-shaped, and located close to the 4th ventricle in the dorsomedial medulla extending from the level of the gracile and cuneate nuclei in the caudal medulla to the facial motor nucleus rostrally. A strong nNOS-immunoreactivity was observed along the solitary tract extending from rostral to caudal medullae. Neuronal cells in gustatory zone of NTS also showed an abundance of nNOS-immunoreactivity (Fig. 1).

Electrophysiological properties

Whole cell recordings were done in 50 neurons of 38 NTS slices from 35 rats (Fig. 2). Neurons examined had a resting membrane potential of more negative than −40 mV, an action potential overshoot greater than 10 mV and an input resistance greater than 100 MΩ. Resting membrane potential of rNTS neurons was −49.2±2 mV, ranged from −35 to −65 mV. Action potential amplitude ranged from 52 to 98 mV (mean: 68.5±2 mV) with a duration measured at half amplitude of 1.8±0.1 ms. Input resistance, determined from the response to a 150 ms, −100 pA hyperpolarizing current pulse, varied between 120 and 840 MΩ (mean: 385±15 MΩ) (Fig. 3). Using a hyperpolarization -depolarization pulse paradigm, neurons in the rNTS were separated into 4 groups. In group I neurons, hyperpolarization altered the repetitive discharge pattern initiated by membrane depolarization to an irregular pattern. In group II neurons, hyperpolarization either delayed the occurrence of the first action potential, or increased the length of the first inter-spike interval in the action potential train produced by membrane depolarization. In group III neurons, hyperpolarization least affected the discharge pattern. In group IV neurons, hyperpolarization shortened the length of the action potential burst produced by membrane depolarization. Some neurons (41%) showed

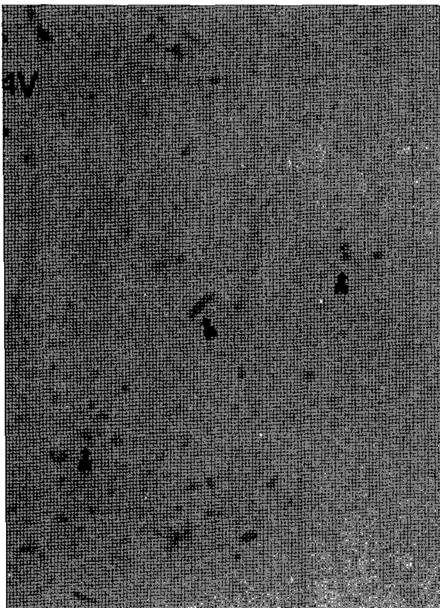

Fig. 1. Neuronal nitric oxide synthase-immunoreactive cells (arrows) in the rostral NTS. Neuronal nitric oxide synthase is demonstrated in neurons along NTS tract. 4V: Fourth Ventricle (n=3).

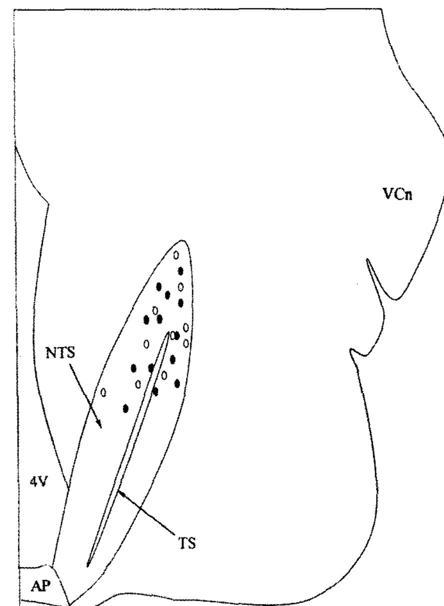

Fig. 2. Schematic diagram of the locations of the neurons of rostral NTS studied in this study. Filled circles indicate the position of neurons which responded to NO donors and open circles indicate the position of unresponsive neurons (AP, area postrema; 4V, fourth ventricle; NTS, nucleus tractus solitarii; TS, tractus solitarius; VCn, ventral cochlear nucleus).

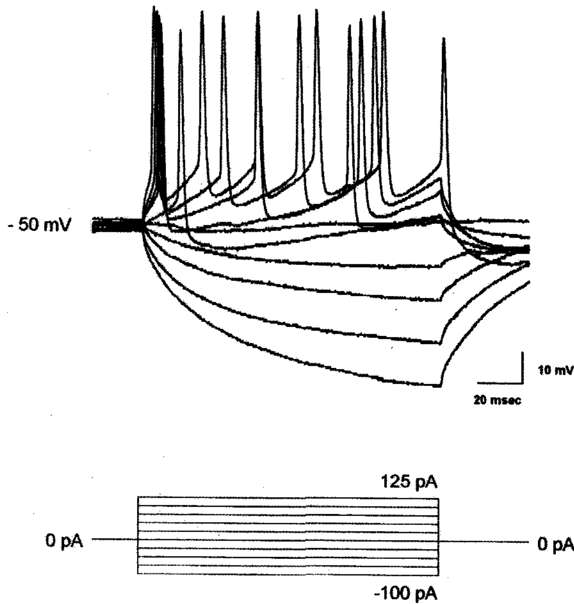

Fig. 3. A representative record of membrane properties and action potential discharge of a gustatory NTS neuron in response to a series of hyperpolarizing and depolarizing current steps. Resting membrane potential was a mean of -49.2 ± 2 mV and action potential amplitude was 68.5 ± 2 mV (n=5).

a spontaneous activity (Fig. 4).

Neuronal responses to NO donors in the rostral NTS neurons

Of neurons recorded in the gustatory NTS, superfusion of SNP (10^{-4} M) or SNAP (10^{-4} M) caused hyperpolarization in 68%, depolarization in 5%, and had no effect in 27%. The responsive neurons were not confined to one region of the gustatory NTS or one neuronal group. Hyperpolarization was accompanied by a decrease in input resistance in 87% of the responsive neurons. In the remaning 13% of the neurons which were hyperpolarized by SNP or SNAP, the input resistance did not change. The duration of the responses always outlasted the duration of the application of SNP or SNAP. SNP or SNAP usually caused neurons to become inactive. The hyperpolarization caused by SNP or SNAP increased the time to initiate the first action potential due to a depolarizing pulse and decreased the number of action potentials elicited during 150 ms depolarizing pulses (Fig. 5). For example, the spike latency during a 100 pA depolarizing pulse was increased by SNP or SNAP. In 5% of the neurons tested, SNP depolarized the membrane potential (data not shown). Depolarization was not always accompanied by increases in input resistance. Under zero current condition, some gustatory NTS neurons were spontaneously active. Superfusion of SNP (10^{-4} M) or SNAP (10^{-4} M) also markedly decreased the number of firing neural discharge of the spontaneous NTS neural activity (Fig. 6).

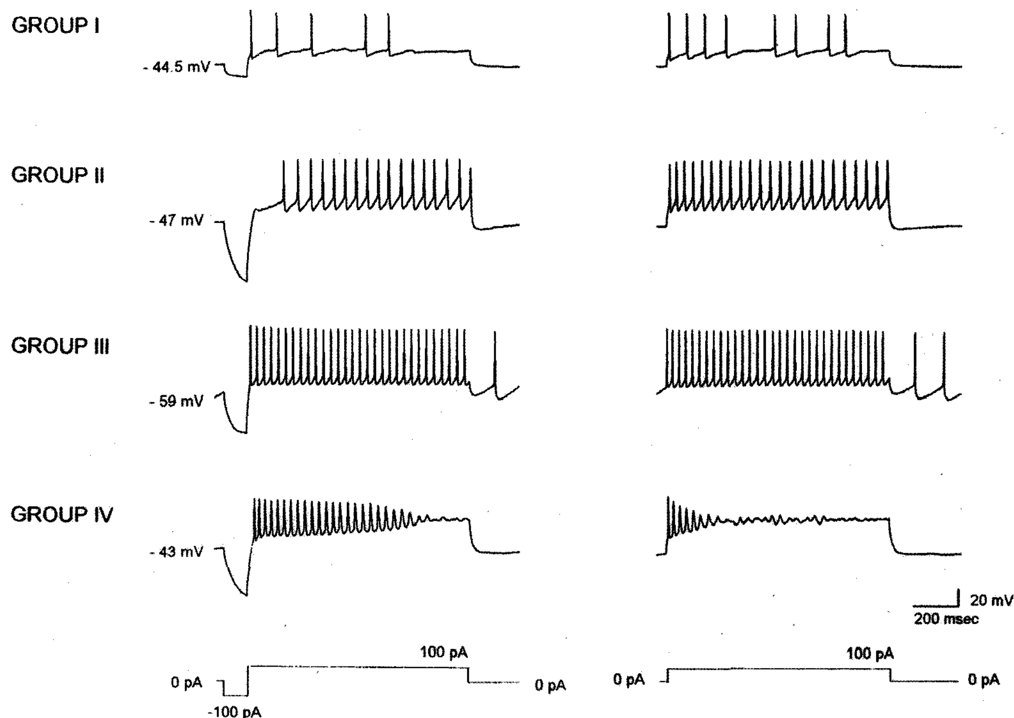

Fig. 4. Repetitive firing discharge patterns of different groups of rNTS neurons to a long (1,200 ms) depolarizing current pulse alone or preceded by a short hyperpolarizing current pulse. Using this current injection protocol, neurons in the rNTS were separated into 4 groups (n=5). The hyperpolarizing pulse disrupts the regular discharge pattern in Group I neurons, causes a long delay in Group II neurons, and has no effects on Group III neurons. Group IV neurons respond to depolarization with a short burst of action potentials.

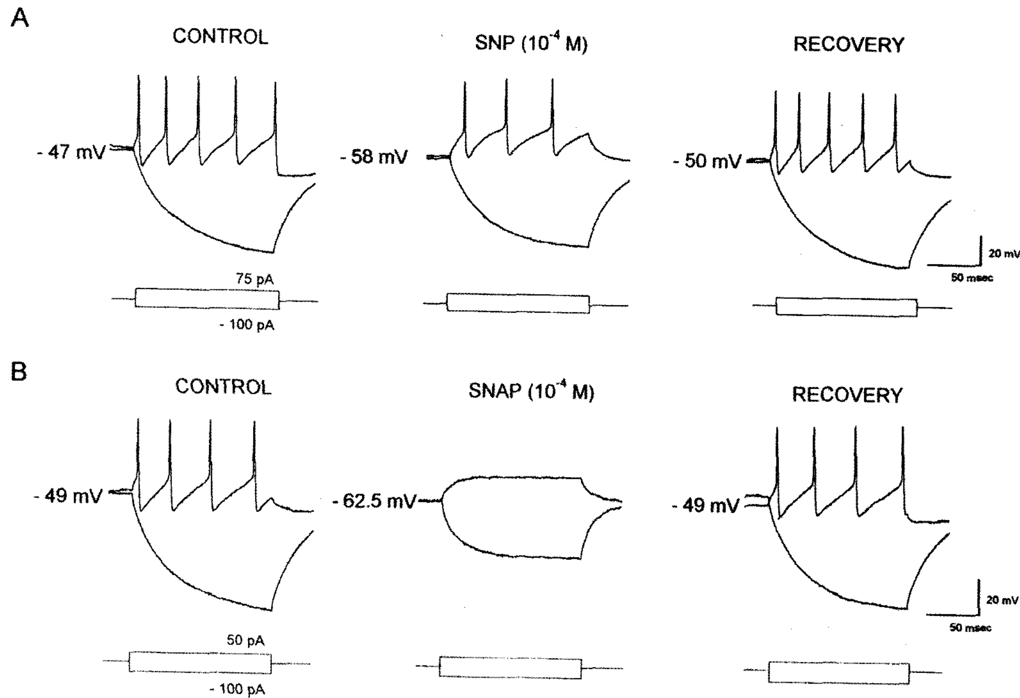

Fig. 5. Responses of a rNTS neuron to a hyperpolarizing and a depolarizing current pulse before, during and after application of SNP (A) or SNAP (B). Notice that NO donors hyperpolarized all of the rNTS neurons, decreased the number of spikes induced by a depolarizing current pulse and decreased the input resistance. The membrane potential at the beginning of each trial is indicated at the left of each trace (n=5).

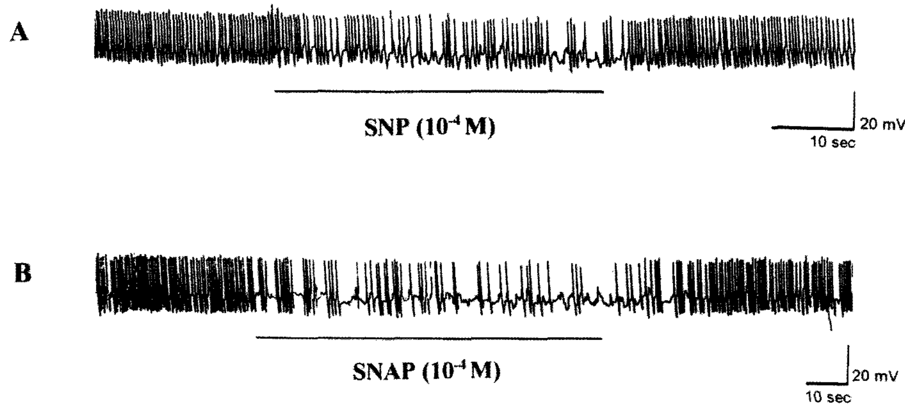

Fig. 6. Effects of SNP (A) or SNAP (B) on the firing neural discharge of the spontaneous rNTS neural activity. Under zero condition, some rNTS neurons spontaneously evoked spikes. Superfusion of NO donors decreased the number of these spontaneous spikes. Horizontal bars below each figure indicate the applicating periods of NO donors (n=5).

Effects of L-NAME on neural activity

To investigate whether endogenous NO were involved in the regulation of neural activity in NTS, effects of L-NAME, a NO synthase inhibitor, on neural responses of NTS by a hyperpolarizing (−100 pA) and a depolarizing current (+50 pA) pulse were examined. Superfusion of L-NAME (10^{-4} M) slightly depolarized the membrane potential and increased the number of action potential during a hyperpolarizing (−100 pA) and depolarizing (+50 pA) current pulses. Input resistance was not altered by superfusion of L-NAME. When L-NAME was washed out with normal PSS, membrane potential and firing rate returned to the normal state (Fig. 7).

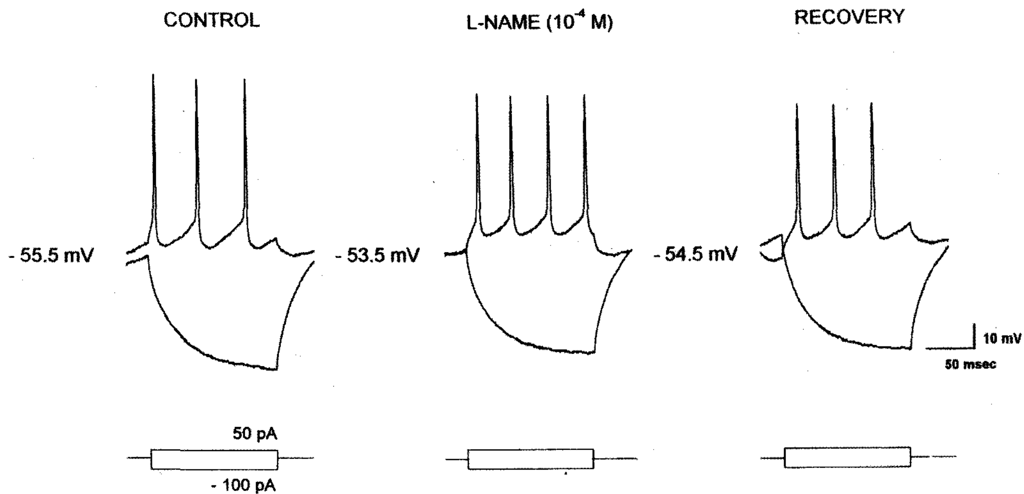

Fig. 7. Effects of L-NAME on neural responses of a gustatory NTS neuron to a hyperpolarizing (−100 pA) and depolarizing (50 pA) current pulse. Superfusion of L-NAME, a NOS inhibitor, slightly depolarized the rNTS neurons and increased the number of spikes. The membrane potential at beginning of each trial is indicated at the left of each trace (n=5).

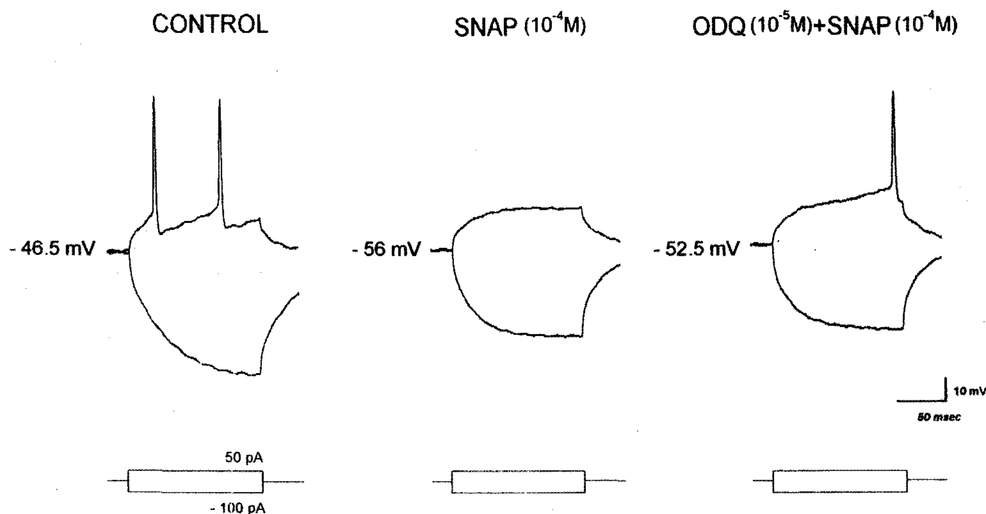

Fig. 8. Effects of ODQ on SNAP-induced neural responses of a rNTS neuron to a hyperpolarizing (−100 pA) and a depolarizing (+50 pA) pulse. Superfusion of ODQ, a soluble guanylate cyclase inhibitor, ameliorated the membrane potential, the input resistance and the firing number decreased by SNAP. The membrane potential at the beginning of each trial is indicated at the left of each trace (n=5).

Effects of ODQ on SNAP-elicited neural responses and neural responses to 8-Br-cGMP

NO mainly exerts its biological effects by activating the soluble isoform of guanylate cyclase to raise the intracellular concentration of cGMP (Verma et al, 1993). Superfusion of ODQ (10^{-5} M), a soluble guanylate cyclase inhibitor, decreased the SNAP-induced changes (Fig. 8). Furthermore, superfusion of 8-Br-cGMP (10^{-5} M), a non-degradable cell-permeable cGMP, hyperpolarized the membrane potential, and decreased the input resistance and the firing rate of action potential in NTS. When 8-Br-cGMP was washed out with normal PSS, membrane potential, input resistance and firing rate returned to the resting value (Fig. 9).

DISCUSSION

Based on repetitive firing properties in response to depolarizing current preceded by a short hyperpolarizing current, neurons of the rNTS in the present study, were classified into 4 groups as in the previous reports (Bradley & Sweazey, 1992; King et al, 1993). Hyperpolarization of

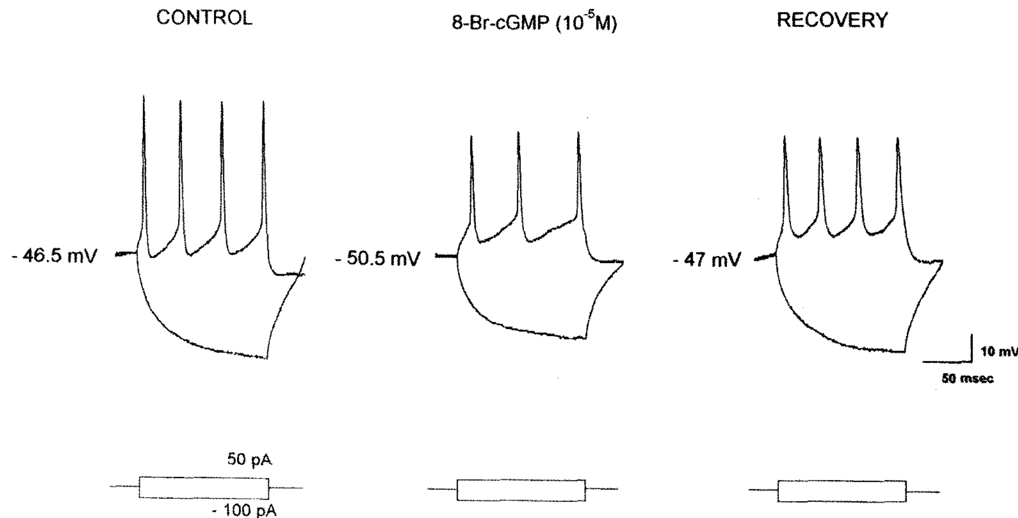

Fig. 9. Responses of a rNTS neuron to a hyperpolarizing (−100 pA) and a depolarizing (50 pA) current pulse before, during and after application of 8-Br-cGMP. 8-Br-cGMP hyperpolarized membrane potential of the NTS neurons and decreased the number of the spikes induced by a depolarizing current pulse (n=5).

Group I neurons altered the repetitive discharge pattern, initiated by membrane depolarization, to an irregular pattern. Hyperpolarization of Group II neurons either delayed the occurrence of the first action potential, or increased the length of the first inter-spike interval of the action potential train produced by membrane depolarization. Hyperpolarization least affected the discharge pattern of Group III neurons. Hyperpolarization of Group IV neurons shortened the length of the action potential burst produced by membrane depolarization. In the present study, Group I, II or III neurons except Group IV were used in investigating the role of NO on regulation of gustatory NTS neural activity, since Group IV neurons were rare.

Resting membrane potentials of rNTS neurons were -49 ± 2 mV. Action potential amplitude was 68.5 ± 2 mV with a duration measured at half amplitude of 1.8 ± 0.1 ms. Input resistance, determined from the response to a 1.5 ms, -100 pA hyperpolarizing current pulse, was 385 ± 15 MΩ. Some rNTS neurons (41%) were spontaneously active.

Recently, NO has been implicated in a wide range of physiological functions such as neurotransmitter or neuromodulator in the nervous system (Dawson et al, 1991). In addition, NOS responsible for NO synthesis was reported to exist in NTS neurons of the rabbit (Gai et al, 1995). The present study showed that neuronal NOS was abundant in neurons of the NTS extending from rostral to caudal medulla. These results may indicate that NO may play a role in regulation of neural activity in gustatory NTS.

However, effects of NO on neuronal activity have been controversial: it has an excitatory or an inhibitory influence in the nervous system. In vagal motoneurons, locus caeruleus and NTS, NO donors increase the neural firing rate, and NOS inhibitors reduce the excitatory effect of NMDA (Travagli & Gillis, 1994; Xu et al, 1994). On the contrary, in the carotid body, rostral or caudal ventrolateral medulla and spinal cord, NO decreases the neural activity (Wang et al, 1994; Hakim et al, 1995; Tseng et al, 1996). In the present study, superfusion of SNP or SNAP, a NO donor, mostly hyperpolarized NTS neurons under depolarizing and hyperpolarizing current injection. The hyperpolarization was mostly accompanied by a decrease in input resistance. The hyperpolarization caused by SNP or SNAP increased the time to initiate the first action potential and decreased the number of action potentials and input resistance during 150 ms depolarizing pulses. SNP or SNAP also markedly decreased the number of firing neural discharge of the spontaneous neural activity under zero current. Furthermore, superfusion of L-NAME, a NOS inhibitor, slightly increased the membrane potential, the input resistance and the firing rate during a hyperpolarizing (−100 pA) and a depolarizing current (+50 pA). These findings suggest that endogenously synthized NO is involved in the regulation of NTS neural activity, playing an inhibitory role to decrease neural activity. The inhibitory effects of NO may not be attributed to a cytotoxic effect of NO (Akira et al, 1994), since the removal of SNP, SNAP or L-NAME rapidly resumed the electrical properties to normal resting state. While SNP or SNAP decreased the activity of most of NTS neurons, no effect was seen in 36% of neurons tested. It is assumed that some neurons in gustatory NTS have no target molecules activated by NO or that neurons positioned within the slices may not have been exposed to the expected concentration of NO donors because of reduced access to the superfused medium.

NO has a stimulatory action on neurotransmitter release (Prast & Philippu, 1992). In addition, it has been reported that NO releases GABA in cerebral cortical neurons and GABA in turn has profound inhibitory effects in the rNTS slice (Wang & Bradley, 1992; Ohkuma et al, 1995). On contrary, NO was also known to have direct postsynaptic inhibitory action (Ohkuma et al, 1998). Therefore, it is not clear in the present study whether inhibitory effects of NO are due to any direct postsynaptic action or indirect presynaptic action.

Since most of the neurons responded to NO by inhibition, NO probably plays a major role in sensory processing by the rNST. Previous investigators have observed that stimulation of the tongue with some taste stimuli occasion-

ally causes inhibition of the ongoing spontaneous activity of second order neurons in the taste pathway, although the predominant result of gustatory stimulation is excitation (Travers et al, 1979; Ogawa et al, 1984). Until recently, NTS was thought to have a relatively straight forward influence on gustatory processing, primarily through excitatory synapses. However, there is a growing body of evidence suggesting that an inhibition has a significant role in sensory processing in the rNST. Halpern & Nelson (1965) recorded from a region of the gustatory NST receiving inputs from both the chorda tympani and glossopharyngeal nerves innervating taste receptors on the anterior and posterior tongue, respectively. When the chorda tympani nerve input was eliminated by local anesthesia, the response to gustatory stimulation of the posterior tongue increased the magnitude, which was interpreted as removal of an inhibitory input. Extracellular recordings from single gustatory neurons receiving convergent input from anterior tongue and posterior oral cavity taste receptors revealed that when both areas are stimulated simultaneously, the response is not a simple summation of the neural responses to separate stimulation of anterior and posterior fields (Sweazey & Smith, 1987). Rather, the response magnitude is in between the response magnitudes evoked by stimulation of the individual fields. The response attenuated by simultaneous stimulation of anterior and posterior receptive fields was hypothesized to be the result of an inhibitory interaction.

Based on results of behavioral experiments in humans there is also evidence of inhibitory interactions between the glossopharyngeal and chorda tympani nerves at the level of the rNST. Damage or anesthesia of the chorda tympani nerve results in taste phantoms (aberrations of perceptual responses to taste stimuli) that disappear if the glossopharyngeal nerve receptive field is anesthetized (Miller & Bartoshuk, 1991). Inhibitory interactions may also occur independently of convergent input derived from the chorda tympani and glossopharyngeal nerve. For example, amiloride, the sodium channel blocker, suppresses whole nerve and single fiber responses of the chorda tympani to NaCl (Brand et al, 1985; Formaker & Hill, 1988). When responses of the second order neurons in rNST were examined after application of amiloride to the tongue, the responses of the central neurons changed (Giza & Scott, 1991). Many NTS neurons increased their firing rates following the application of amiloride, suggesting that removal of the amiloride-sensitive afferent input disinhibits these neurons (Nuding & Frank, 1992). Therefore, the present study suggests that inhibition plays an important role in sensory processing by rNTS neurons, and that afferent input can be altered or modulated by the inhibitory action.

NO exerts its role through binding the heme of intracellular soluble guanylate cyclase, the enzyme that synthesizes cGMP (Schmidt et al, 1993), although it has been also known to modulate various ion channels directly in the other organ or tissues such as cardiovascular tissues, gastrointestinal tract and nervous system (Robertson et al, 1993; Fagni & Bockaert, 1996; Rao et al, 1996). In the present study, ODQ, a soluble gunaylate cyclase inhibitor, inhibited the SNP-induced hyperpolarization, input resistance and firing rate. Furthermore, 8-Br-cGMP, a nongradable membrane-permeable cGMP, increased the membrane potential, the input resistance and the firing rates in NTS under current injection from −100 to +50 pA. These results suggest that NO functions by activating soluble guanylate cyclase, and increasing cytosolic cGMP concentration in the gustatory NTS. cGMP can bind to at least three distinct classes of proteins. First, cGMP can bind to gate ion channels directly. Cyclic nucleotide-gated channels have been found in both the CNS and PNS (Goulding et al, 1992). Second, cGMP can activate cGMP-dependent protein kinase (Scott, 1991). Finally, two subtypes of phosphodiesterases (PDE) are known to be regulated by cGMP. Type II PDE (GS-PDE) is stimulated by cGMP, whereas type III PDE (GI-PDE) is inactivated when cGMP is bound (Beavo et al, 1971a,b). Among these possible cGMP subpathways, a question of which mechanism is involved in regulating the NTS neuronal activity in the future needs to be investigated.

In summary, endogenously-derived NO decreases neural activity of gustatory NTS by activating soluble guanylate cyclase.

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