Tetanic Stimulation Induces Short-Term Potentiation of Inhibitory Synaptic Activity in the Rostral Nucleus of the Solitary Tract

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Grabauskas, Gintautas and Robert M. Bradley. Tetanic stimulation induces short-term potentiation of inhibitory synaptic activity in the rostral nucleus of the solitary tract. J. Neurophysiol. 79: 595–604, 1998. Whole cell recordings from neurons in the rostral nucleus of the solitary tract (rNST) were made to explore the effect of high-frequency tetanic stimulation on inhibitory postsynaptic potentials (IPSPs). IPSPs were elicited in the rNST by local electrical stimulation after pharmacological blockade of excitatory synaptic transmission. Tetanic stimulation at frequencies of 10–30 Hz resulted in sustained hyperpolarizing IPSPs that had a mean amplitude of –68 mV. The hyperpolarization resulted in a decrease in neuronal input resistance and was blocked by the γ-aminobutyric acid-A (GABA_A) antagonist bicuculline. For most of the neurons (n = 87/102), tetanic stimulation resulted in a maximum hyperpolarization immediately after initiation of the tetanic stimulation, but for some neurons the maximum was achieved after three or more consecutive shock stimuli in the tetanic train of stimuli. When the extracellular Ca^2+ concentration was reduced, the maximum IPSP amplitude was reached after several consecutive shock stimuli in the tetanic train for all neurons. Tetanic stimulation at frequencies of 30 Hz and higher resulted in IPSPs that were not sustained but decayed to a more positive level of hyperpolarization. In some neurons the decay was sufficient to become depolarizing and resulted in a biphasic IPSP. It was possible to evoke this biphasic IPSP in all the neurons tested if the cells were hyperpolarized to –75 to –85 mV. The ionic mechanism of the depolarizing IPSPs was examined and was found to be due to an elevation of the extracellular K^+ concentration and accumulation of intracellular Cl^−. Tetanic stimulation increased the mean 80-ms decay time constant of a single shock–evoked IPSP up to 8 s. The length of the IPSP decay time constant was dependent on the duration and frequency of the tetanic stimulation as well as the extracellular Ca^2+ concentration. Afferent sensory input to the rNST consists of trains of relatively high-frequency spike discharges similar to the tetanic stimulation frequencies used to elicit the IPSPs in the brain slices. Thus the short-term changes in inhibitory synaptic activity in the slice preparation probably occur in vivo and may play a key role in taste processing by facilitating synaptic integration.

INTRODUCTION

The rostral nucleus of the solitary tract (rNST) is the site of the first central synapse of afferent fibers innervating taste buds and somatosensory receptors of the oral cavity (Bradley et al. 1985; Hamilton and Norgren 1984; Hanamori and Smith 1989). These afferent fibers, traveling in the intermediate (VII) and glossopharyngeal (IX) nerves, form the solitary tract (ST) and make excitatory synapses with rNST neurons (Grabauskas and Bradley 1996; Wang and Bradley 1995). Recently, we examined the characteristics of the postsynaptic potentials at this synapse using intracellular recording in a horizontal brain slice preparation of the rat rNST. The postsynaptic potentials elicited by electrical stimulation of the ST have a complex waveform, resulting from a mixture of both excitatory and inhibitory postsynaptic potentials (Grabauskas and Bradley 1996). Thus both excitation and inhibition are involved in synaptic transmission at the first central synapse in the taste pathway. Although the role of excitation at this synapse seems clear, the role of inhibition is less obvious because most taste stimuli excite second-order neurons and only a few examples of inhibition by gustatory stimuli have been reported (Travers and Smith 1979). However, there is now physiological, pharmacological, and anatomic evidence indicating that inhibition mediated by γ-aminobutyric acid (GABA) has a major influence on synaptic processing in the rNST. For example, investigators using immunocytochemical techniques have revealed that a major population of rNST neurons are GABAergic (Davis 1993; Lasiter and Kachele 1988), and in electrophysiological studies in vitro rNST neurons have been shown to respond to GABA (Liu et al. 1993; Wang and Bradley 1993, 1995). The present series of experiments was designed to explore in more detail the mechanisms of inhibition in rNST.

In our previous studies of inhibition in rNST, we used single shock stimuli to generate inhibitory postsynaptic potentials (IPSPs). However, afferent gustatory input to the rNST typically consists of trains or burst of impulses, rather than isolated action potentials, at frequencies ranging from 1 to 60 Hz, depending on the stimulus modality and concentration (Fishman 1957; Frank et al. 1988; Ogawa et al. 1968, 1973). In addition, there is considerable convergence at the synapse between the afferent input and the second-order rNST neurons, so that response frequencies are 4.3 times higher than responses recorded in primary afferent taste fibers (Doetsch and Erickson 1970; Vogt and Mistretta 1990). Thus the second-order neuron in the taste pathway would normally receive a high frequency of input when taste stimuli are flowed over the tongue.

In the present study we have used high-frequency tetanic stimulation to mimic the normal frequency of impulses arriving at the rNST neurons. In addition, to limit our investigations to IPSPs, we used glutamate receptor blockers to eliminate excitatory potentials. IPSPs were then evoked by direct electrical stimulation of inhibitory interneurons in the vicinity of the ST (Grabauskas and Bradley 1996).

METHODS

Slice preparation

Male and female rats, 3–4 wk old, were used. Although these are young rats, we recently have demonstrated that the intrinsic
properties of rNST neurons have reached mature values by this age (Bao et al. 1995). The preparation of horizontal rNST brain slices has already been described in detail (Bradley and Sweazey 1992; Grabauskas and Bradley 1996). Briefly, rats were anesthe-
titized with pentobarbital sodium (50 mg/kg) and decapitated. The whole brain, including the cervical spinal cord, was rapidly re-
moved and placed in ice-cold physiological saline containing (in mM) 124 NaCl, 2.5 KCl, 2.5 CaCl2, 1.3 MgSO4, 26 NaHCO3, 1.25 KH2PO4, and 25 glucose, gassed with 95% O2-5% CO2 to give a pH of 7.3. The brain was transected at the level of the pons and just below the obex and the cerebellum removed. Horizontal 300-μm slices containing the whole NST were cut on a Vibratome and placed in a holding chamber. After at least 1 h recovery, the slice containing the NST was transferred to the recording chamber (volume of 1 ml), where it was submerged and held in place by a net and continuously superfused (2 ml/min) with physiological saline at room temperature. Because the PSPs elicited by electrical stimulation are mixed excitatory and inhibitory potentials once the basic properties of a rNST neuron was established, we performed all further experiments in the presence of 50 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) to block the excitatory component of the PSPs.

FIG. 1. A: recording of a sustained hyperpolarizing inhibitory postsynaptic potential (IPSP) in a rostral nucleus of the solitary tract (rNST) neuron in response to tetanic stimulation in the presence of 20 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 50 μM n-2-amino-5-phosphonovalerate (APV; top trace). The sustained hyperpolarization amplitude was −71 mV. After the tetanic stimulation was terminated, the cell membrane potential returned to its resting level with a prolonged time constant. Application of 100 μM bicuculline methiodide (BMI) completely blocked the tetanic stimulation evoked IPSP (bottom trace). B: change in input resistance during tetanic stimulation. During tetanic stimulation the input resistance of the recorded cell decreased by 50%. After the tetanic stimulation was stopped, the input resistance of the recorded cell returned to control levels with a time course similar to the decay time of IPSP. Input resistance was measured by applying −100-pA, 100-ms current steps. The amplitudes of negative potentials deflections correspond to the input resistance changes of the recorded cell. Horizontal bar indicates duration of tetanic stimulation at 50 Hz.

FIG. 2. A: time course of the development of the tetanically evoked IPSPs. For some rNST neurons the maximum IPSP amplitude was achieved after several consecutive stimuli of the tetanic stimulus train (left), whereas for other neurons the maximum IPSP amplitude was achieved at the beginning of the tetanic stimulation (right). B: influence of extracellular Ca2+ concentration on the development of the tetanically evoked IPSPs. Although the extracellular Ca2+ concentration had no effect on the final amplitude of the tetanically evoked IPSPs, it did influence the time course of the development of the IPSPs. When the extracellular Ca2+ was 2.5 mM, the maximum IPSP amplitude was reached after the 1st shock of the tetanic stimulation. When the extracellular Ca2+ was 0.3 mM, the maximum IPSP amplitude was reached after several shocks (11) of the tetanic stimulation. Both intracellular recordings obtained at the same stimulus strength.

The resulting IPSPs were evoked by direct electrical stimulation of rNST inhibitory interneurons (Glaum and Brooks 1996; Grabauskas and Bradley 1996). In 12 experiments we used a N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES)—buffered solution to elucidate the role of HCO3− ions in the inhibitory synaptic transmission. In these experiments with HCO3−-free physiological saline, the NaHCO3 was replaced by 10 mM HEPES, and 16 mM Na2C4H4O4 (sodium succinate) and gassed with O2. The pH was adjusted with NaOH to 7.4. To ensure that the HCO3−/CO2−-buffered solution was completely exchanged with the HCO3−-free (HEPES buffered) perfusing solution, in four experiments the slices were first incubated in the HCO3−-free saline, and then switched to the HCO3−/CO2−-buffered perfusing solution. In experiments examining the effects of different concentrations of extracellular Ca2+ and K+ ions on the IPSPs, the ion concentration in the perfusing solution was reduced by isomolar substitution of NaCl. Even though the volume of the slice chamber (1 ml) was small enough to allow for rapid exchange of contents, we waited for >5 min before making further recordings to allow the cell to stabilize after perfusing solutions were changed.

Patch pipettes, pulled in two stages from 1.5 mm OD borosilicate filament glass, were filled with a solution containing (in mM) 130 K-glucuronate, 10 HEPES, 10 ethylene glycol-bis(β-aminoethyl ether)-N,N,N’,N’-tetraacetic acid, 1 MgCl2, 1 CaCl2, 2 ATP, and 0.2 GTP. Pipette solutions were adjusted to a pH 7.2–7.3 with...
KOH and had an osmolarity of 275–292 mosM. Electrode resistance was between 5 and 8 MΩ. Electrodes were positioned under visual control over the medial rNST in an area extending from 1.5 to 2.5 mm rostral to the obex.

**Drug application**

Drugs were applied to the slice by switching the normal physiological saline to one containing known concentrations of the drug. Drugs used in these experiments were bicuculline methiodide (BMI) obtained from Sigma, and 2-hydroxysaclofen (OH-saclofen) supplied by Research Biochemicals International. The concentrations of the different drugs used were those we have used in our previous studies of rNST neurons and were 400 μM for OH-saclofen and 20 or 100 μM for BMI (Wang and Bradley 1993, 1995).

**Stimulation procedure**

Postsynaptic potentials (PSPs) were elicited by delivery of stimuli (0.1 ms duration) via a bipolar stimulating electrode consisting of tightly twisted pairs of 70-μm-diam, Teflon-insulated, platinum wires (200 μm overall diameter, including insulation, 140 μm diameter metal stimulating surface) placed under direct visual control in the region of the solitary tract in rostral (2.5–3.0 mm rostral to the obex) or intermediate (−1.5 mm rostral to the obex) portions of rNST. The intensity of the stimulus was adjusted to evoke IPSPs and ranged from 0.1 to 3.0 mA. The distance between the stimulating electrode and recording site was between 0.5 and 1.0 mm. In some experiments single shock stimuli were used, and in other experiments tetanic trains of stimuli lasting between 50 ms and 5 s at frequencies ranging from 10 to 100 Hz were used.

**Data analysis**

Recordings were made using an Axoclamp 2B amplifier (Axon Instruments) in current-clamp mode. Bridge balance was carefully monitored throughout the experiments and adjusted when necessary. The junction potential due to K gluconate (10 mV) was subtracted from the recorded membrane voltages (Standen and Stanfield 1992). Recordings were considered acceptable if the resting membrane potential was greater than −50 mV, action-potential amplitude was >50 mV, and neuron input resistance was >300 MΩ. The time constants of the IPSPs were measured by fitting a single-exponential function.

All data were stored and analyzed using pCLAMP software (Axon Instruments). Data were plotted and linear regression analysis performed using Microcal Origin software. The numerical values are given as means ± SE.

**RESULTS**

The results are based on recordings from 102 neurons in 88 slices taken from 63 rats. The basic characteristics of all the neurons were measured in control physiological saline. Resting membrane potentials were between −50 and −68 mV (−56 ± 0.8 mV, mean ± SE). Overshooting action-potential amplitudes ranged from 52 to 95 mV (65 ± 1.65 mV) with a mean duration measured at half-amplitude of 2.5 ± 0.18 ms. Input resistance, determined from the steady-state portion of the response to a 100 ms, −100 pA hyperpolarizing current pulse varied between 330 and 800 MΩ (463 ± 25 MΩ). Thirty percent of the recorded cells were spontaneously active, generating action potentials at a frequency 0.5–7 Hz. Application of the glutamate receptor antagonists CNQX and APV resulted in a hyperpolarization from their resting membrane potential by 1–5 mV (2.2 ± 0.2 mV) indicating a small tonic glutaminergic input to the neurons.

Single shock stimuli delivered to the rNST in the presence of CNQX and APV resulted in hyperpolarizing IPSPs, which were blocked by 20 μM BMI and were therefore considered to be “pure” IPSPs resulting from activation of presynaptic GABAergic interneurons. Single shock–evoked IPSPs were recorded for all neurons in the study and had a mean latency of 6.8 ± 0.4 ms, an amplitude of up to 18 mV, a rising phase time constant of 6.4 ± 0.2 ms, and a decay phase time constant of 80 ± 5.0 ms.

In contrast to single shock–evoked IPSPs, tetanic stimulation lasting 0.1–5 s at frequencies of 10–30 Hz resulted in hyperpolarizing IPSPs that were maintained at a sustained level for the duration of the stimulus. The mean amplitude

![Graph](http://jn.physiology.org/DownloadedFrom)
FIG. 4. A: influence of membrane potential on the characteristics of the tetanically evoked IPSP in an extracellular solution buffered with HCO$_3$/CO$_2$ (left) and an extracellular solution buffered with N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES; right). In the bicarbonate buffer at the neuron's resting membrane potential ($-58$ mV), the tetanically evoked IPSP decayed back toward the resting membrane potential before the termination of the tetanic stimulation. When the cell was hyperpolarized to $-79$ mV, the IPSP response had a biphasic character. Hyperpolarization of the neuron to $-91$ mV resulted in a purely depolarizing IPSP. Similar results were obtained in the HEPES-buffered extracellular perfusate. In this perfusate the responses to tetanic stimulation were of a smaller IPSP amplitude. Horizontal bar indicates a 5-s tetanic stimulation at 50 Hz. B: plots of the IPSP amplitude at the initiation and termination of the tetanic stimulation when the cell was exposed to a HCO$_3$/CO$_2$ (●, initiation; ▲, termination) and a HEPES (●, initiation; ▲, termination) buffered extracellular solution at different membrane potentials. The reversal potential of the IPSP in either the HCO$_3$/CO$_2$ or HEPES buffer was the same. C: exposure of the neuron to a HCO$_3$-free solution (right) reduced the amplitude of the single shock-evoked IPSP when compared with the single shock IPSP recorded in HCO$_3$-containing solution (left). The reduction of the IPSP amplitude was not accompanied by a change of the IPSP reversal potential. D: relationship between the IPSP amplitudes and the neuron membrane potential. The reversal potential of the single shock-evoked IPSP was $-89$ mV in both the HCO$_3$-free solution and in HCO$_3$-containing extracellular solution.

The time course of the rising phase of the hyperpolarization evoked by tetanic stimulation was of two different types. When the single shock-evoked IPSP amplitude was relatively small ($1-5$ mV), each IPSP evoked by tetanic stimulation summed with the preceding IPSP by a process of synaptic facilitation.

For these “small” amplitude IPSPs, the magnitude of the IPSP increased progressively and reached a maximal level of hyperpolarization after the first three to nine shocks in the tetanic train when the tetanic stimulation frequency was 30 Hz. The final amplitude of the hyperpolarization level was up to six times greater than the amplitude of the small IPSP evoked by a single shock (Fig. 2A, left). In contrast, when the single shock-evoked IPSP was of a “high” amplitude ($5-18$ mV, $n = 87$), the hyperpolarization achieved a maximum or close to maximum value immediately after initiation of the tetanic stimulation (Fig. 2A, right).

It has been suggested that a residue of Ca$^{2+}$ that enters the nerve terminal during the nerve impulse is responsible for this
synaptic facilitation. The residual Ca$^{2+}$ combines with Ca$^{2+}$ that enters the presynaptic terminal at the time of a second nerve impulse, giving rise to an increased release of transmitter by the second impulse (Katz and Miledi 1968). We examined the role of Ca$^{2+}$ in the synaptic facilitation resulting from tetanic stimulation by reducing the extracellular Ca$^{2+}$ concentration from 2.5 to 0.3 mM ($n = 12$). The amplitude of the IPSP was reduced by the lowered external Ca$^{2+}$ concentration, and the facilitation was then observed in all the tested neurons. However, the 0.3-mM external Ca$^{2+}$ did not influence the maximum amplitude of the hyperpolarization (Fig. 2B). Thus the reduced external Ca$^{2+}$ concentration influences the rate of synaptic facilitation, but not the sum of the IPSPs resulting from tetanic stimulation.

Tetanic stimulation at frequencies of 30 Hz and higher resulted in a more complex postsynaptic response. The hyperpolarization was not sustained during the stimulation period, and decayed to a more positive level of hyperpolarization. Depending on the cell resting membrane potential, the hyperpolarization could decay sufficiently to become depolarizing, resulting in a biphasic IPSP. Several parameters influenced the characteristics of the decay phase of the hyperpolarization, including stimulus frequency, stimulus amplitude, and the cell resting membrane potential. The decay of the sustained hyperpolarization began 0.1–1.8 s after initiation of the tetanic stimulation.

For most neurons tested, the amplitude of the decay in the hyperpolarization reached a plateau phase that was $9 \pm 0.9$ mV positive to the maximal IPSP amplitude (Fig. 3A). However, in a few neurons ($n = 5$) the tetanic response was biphasic, the hyperpolarization decayed back to the resting membrane potential and then became depolarizing, evoking a burst of action potentials (Fig. 3B). The rate of the decay of the hyperpolarization was stimulus frequency dependent, increasing with higher tetanic frequencies (Fig. 3B). It was possible to evoke this biphasic response in all the neurons tested if the cells were hyperpolarized by intracellular current injection to $-75$ to $-85$ mV. At levels of hyperpolarization more negative than $-85$ mV, the tetanic response consisted of a purely depolarizing IPSP (Fig. 3B). When the IPSP amplitude at the beginning (■) and end (●) of the tetanic stimulation was plotted at different membrane potentials, the beginning IPSP amplitude reversed at $-85$ mV, and the final IPSP amplitude was $2–25$ mV more positive than the initial IPSP amplitude (Fig. 4B). Thus the characteristic of the tetanically induced IPSPs were dependent on the neuron’s membrane potential.

Recently Staley et al. (1995) concluded that HCO$_3^-$ ions are responsible for the generation of the depolarizing phase of GABA$_A$-mediated IPSPs in hippocampal neurons. We therefore examined the role of HCO$_3^-$ ions in the biphasic

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**FIG. 5.** Effect of different external concentrations of K$^+$ on the characteristics of the single shock and tetanically evoked IPSP. **A:** at higher concentrations of the external K$^+$, the single shock–evoked (↑) IPSP reversal potential was shifted to a more positive value. The IPSP reversal potential was $-95$ mV for 1 mM, $-89$ mV for 2.5 mM, and $-72$ mV for 10 mM external concentrations of K$^+$. Membrane potential was manipulated by current injection into the neuron. **B:** relationship between the amplitude of the single shock–evoked IPSPs and the neuron’s membrane potential when exposed to different extracellular K$^+$ concentrations. **C:** effect of different external concentrations of K$^+$ on the characteristics of a tetanically evoked IPSP recorded at different membrane potentials. In the presence of 2.5 mM external K$^+$, the IPSP amplitude was reduced from its initial amplitude by $12–15$ mV at different membrane potentials (left). In presence of 10 mM external K$^+$, the reduction of the IPSP from its initial amplitude was $3–8$ mV. In addition, the depolarizing IPSP occurred at a more positive membrane potential when the neuron was exposed to a 10-mM external K$^+$. Horizontal bar indicates a 5-s tetanic stimulation at 50 Hz. **D:** plots of the IPSP amplitude at the initiation and termination of the tetanic stimulation when the neuron was exposed to 2.5 mM (●, initiation; ■, termination) and 10 mM (■, initiation; ●, termination) external K$^+$. 

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FIG. 6. Influence of tetanic stimulus frequency and duration on the decay time of the IPSP in control and low Ca\(^{2+}\) concentration perfusing solution. A: the duration of the decay time of the IPSP increases with increasing frequency of the tetanic stimulation. Arrows indicate the single shock–evoked IPSP before and after tetanic stimulation. Bar indicates tetanic stimulus duration. B: relationship between the frequency of the tetanic stimulus and the decay time constant of the IPSP. All measurements are made with a 1-s duration tetanic stimulus. The decay time of the IPSP also depends on Ca\(^{2+}\) concentration in the perfusing solution. With low (0.3 mM) extracellular Ca\(^{2+}\), the decay time constants are shorter than decay time constants measured in control extracellular solutions. Values are means ± SE. C: the duration of the decay time of the IPSP increases with increasing duration of the tetanic stimulation at a constant frequency. In spontaneously active neurons, higher values of the decay time constant of the IPSP also result in an increasing delay of the occurrence of the 1st spike after termination of the tetanic stimulus. Bar indicates the stimulus duration at 30 Hz frequency. D: relationship between duration of the tetanic stimulus and the length of the decay time constant of the IPSP. All measurements were obtained at 30 Hz. The decay time of the IPSP is also depended on Ca\(^{2+}\) concentration in the perfusing solution. A lower extracellular Ca\(^{2+}\) concentration results in shorter decay time constants than those measured in control extracellular solutions. Values are means ± SE. E: the decay time constant of the tetanically evoked IPSP depends on the extracellular Ca\(^{2+}\) concentration. For spontaneously active neurons, it also delays the occurrence of the 1st spike after termination of the tetanic stimulus (left). However, the decay time as well as the occurrence of the 1st spike is markedly shorter when the extracellular Ca\(^{2+}\) concentration is lowered (right). Horizontal bars indicate 1 s tetanic stimulation at different frequencies.

depolarizing IPSPs in rNST neurons. Superfusion of the slices with a HCO\(_3^-\)-free solution resulted in 10–50% reduction of the single shock evoked IPSP amplitude (Fig. 4C, right) compared with neurons in bicarbonate superfusate (Fig. 4C, left), but had no effect on the IPSP reversal potential (Fig. 4D). When the amplitude of the initial (▲) and final (▼) IPSP resulting from tetanic stimulation was measured in HCO\(_3^-\)-free superfusate, the initial amplitude was reduced by 10–35%, but there was no consistent effect on either the time course, amplitude of the decay phase, or the reversal potential of the IPSP (n = 12; Fig. 4A, right, and Fig. 4B).
The external K\(^+\) concentration has also been shown to alter both IPSP amplitude and reversal potential in hippocampal slices (Thompson et al. 1988; Thompson and Gähwiler 1989). We examined the effect of different extracellular K\(^+\) concentrations (1, 2.5, and 10 mM) on the IPSP amplitude and reversal potential during single shock and 1–5 s duration tetanic stimulation \((n = 10)\). Single shock stimulation at various membrane holding potentials revealed that the external K\(^+\) concentration influenced the reversal potential of the IPSP. The reversal potential of single shock–evoked IPSPs was \(-93 \pm 1.1\) mV \((n = 8)\) for 1 mM external K\(^+\), \(-87 \pm 0.6\) mV \((n = 25)\) for 2.5 mM external K\(^+\), and \(-71 \pm 1.3\) mV \((n = 10)\) for 10 mM external K\(^+\). These results indicate that elevation of the external K\(^+\) concentration shifted the reversal potential of the IPSPs in a positive direction (Fig. 5, A and B). However, application of an external solution containing 1 mM K\(^+\) resulted in a 0.5- to 2.0-mV membrane hyperpolarization, and superfusion with 10 mM K\(^+\) resulted in membrane depolarization by 6–9 mV when compared with the resting membrane potential recorded in the control superfusate (2.5 mM K\(^+\)). Tetanic stimulation in the presence of 10 mM external K\(^+\) revealed that the difference between the initial and final IPSP amplitude (■ and ○) was 45–90% smaller \((n = 15)\) when compared with the difference between the initial and final IPSP amplitude (▲ and ▼) in control superfusate (2.5 mM K\(^+\); Fig. 5C). In addition, for the 10-mM external K\(^+\) solution, the biphasic pattern of response occurred at more positive membrane potentials than in control superfusate (−55 to −70 mV, \(n = 8,\) Fig. 5, C and D).

Depending on the frequency of the tetanic stimulation, the time course of the decay of the IPSP was prolonged. Tetanic stimulation increased the mean 80-ms decay time constant of the single shock–evoked IPSP up to 8 s for a 100-Hz tetanic stimulation (Fig. 6, A and B). In addition, increasing the length of the tetanic stimulus duration resulted in an increase in the IPSP decay time constant (Fig. 6, C and D). Prolongation of the decay time also delayed the occurrence of action-potential generation in spontaneously active neurons (Fig. 6, C and E). The delay time between termination of the tetanic stimulation and the occurrence of the first spike was approximately double the time constant of the IPSP decay time. The external Ca\(^{2+}\) concentration also influences the length of the tetanically induced IPSP decay time. The length of the IPSP decay time decreased when the external Ca\(^{2+}\) concentration was reduced to 0.3 mM) by 74–90% of the values measured in the control superfusing solution (Fig. 6, B, D, and E). Because the time constant of the IPSP decay phase was influenced by the concentration of external Ca\(^{2+}\), the underlying mechanism of the changes in the length of the IPSP decay phase probably results from calcium accumulation in the presynaptic terminals. The calcium accumulation during the sustained stimulation prolongs the transmitter release as described by Katz and Miledi (1968).

Deisz and Prince (1989) have suggested that GABA is capable of acting on presynaptic GABA\(_B\) receptors to decrease its own evoked release. It is possible that this presynaptically controlled reduction in GABA release may be responsible for the decay of the hyperpolarization during tetanic stimulation. We therefore used the GABA\(_B\) receptor antagonist OH-saclofen to block the GABA\(_B\) receptors. Application of 400 μM OH-saclofen \((n = 8)\) did not prevent the decay of the hyperpolarization during the tetanic stimulation, indicating that presynaptic GABA\(_B\) receptors do not play a role in the decay process in rNST (Fig. 7).

**DISCUSSION**

Tetanic stimulation at frequencies mimicking the normal input to rNST neurons resulted in marked short-term changes in the IPSPs recorded from these cells. The tetanically induced IPSPs were blocked in the presence of the GABA\(_B\) antagonist bicuculline and were therefore mediated by GABA. The time course of the tetanically induced IPSPs was increased when compared with single shock–induced IPSPs, and the length of the decay time constant of the IPSPs was dependent on both the duration and the frequency of the tetanic stimulation as well as the concentration of extracellular Ca\(^{2+}\). In addition, tetanic stimulation could also elicit a biphasic response consisting of an initial hyperpolarizing potential followed by a late depolarizing potential sufficient to initiate action potentials. Similar biphasic inhibitory responses have previously been reported to occur in the hippocampus and are reported here for the first time in rNST. Thus high-frequency afferent input to the rNST is capable of modifying the inhibitory synaptic activity that would consequently influence the processing of sensory information. With the use of a totally different experimental approach, tetanic stimulation has recently been shown to have a marked long-term effect on inhibitory synaptic activity in the caudal, nongustatory, NST as well (Glaum and Brooks 1996).

When we began these experiments, we observed that although some of the postsynaptic cells responded to tetanic stimulation by progressively increasing hyperpolarizing amplitudes, others responded with a maximum IPSP amplitude after the very first shock of the tetanic stimulus train. However, when the extracellular Ca\(^{2+}\) level was lowered, all the tested neurons reached maximum hyperpolarization after five or more stimuli of the tetanic stimulation. This can be explained by assuming that the GABA released was suffi-
cient to bind all the available GABAergic receptors at the initiation of the tetanic stimulus in some cells. For those neurons that responded with progressively increasing hyperpolarizing levels, the concentration of GABA sufficient to activate all the available receptors was achieved after several consecutive shocks of the tetanic stimulus. By lowering the extracellular calcium level, the quanta of transmitter released per stimulus shock was reduced, causing neurons that normally reached a maximum hyperpolarizing level after the first shock to require several consecutive shocks to achieve the maximum level.

The posttetanic potentiation of the IPSP decay phase was relatively short lasting (<8 s) and was dependent on the frequency and duration of the tetanic stimulus. However, even though the posttetanic potentiation was short lasting, it was sufficient to suppress action-potential generation in spontaneously active neurons. Although investigators have reported that rNST neurons are spontaneously active in vivo (Hill et al. 1983), no functional significance has been attached to this activity. It is possible that spontaneous activity may serve to release a chronic level of inhibitory neurotransmitter, and the prolonged decay phase that follows tetanic stimulation, by suppressing the spontaneous activity, may serve to eliminate the chronic inhibition and therefore indicate the presence of afferent information. Thus the prolonged posttetanic decay phase of the IPSP may be significant in the transmission of sensory information.

Ionic mechanisms of the biphasic IPSPs

At tetanic frequencies above 30 Hz, the amplitude of the hyperpolarizing IPSPs were not maintained and for several cells became biphasic with an initial hyperpolarization followed by a slow depolarization. These biphasic IPSPs in response to high-frequency (100–200 Hz) stimulation or by application of a high concentration of GABA have been reported to occur in the hippocampus by a number of investigators but either do not occur in other brain areas or have not been systematically investigated (see Lambert and Grover 1995).

The initial reduction of the hyperpolarizing component of the IPSP could result from either a presynaptic or postsynaptic mechanism. There are at least two presynaptic mechanisms that may mediate a decrease in amplitude of the IPSP. The first mechanism is a transient decrease in the quantal content of released neurotransmitter due to a variety of mechanisms, such as a lack of available neurotransmitter vesicles that are biochemically prepared to be secreted, or decrease in the number of ‘empty’ active zones to which vesicles can fuse and subsequently secrete (Korn et al. 1982). Although this could explain the initial decrease in the hyperpolarization, it cannot explain the depolarizing component of the IPSPs. The second mechanism proposed for the decreased IPSP amplitude is a presynaptic one resulting from autoinhibition of GABA release due to activation of presynaptic GABA<sub>A</sub> receptors (Davies et al. 1990; Deisz and Prince 1989). However, experiments in which we blocked the GABA<sub>A</sub> receptors using OH-saclofen had no effect on the IPSPs, indicating that a presynaptic mechanism was probably not involved in the rNST neurons.

Several postsynaptic mechanisms have been suggested to explain why the hyperpolarizing IPSPs were not maintained but became depolarizing. For example, it has been shown that hippocampal GABA receptors desensitize after prolonged GABA application (Numann and Wong 1984; Wong and Watkins 1982). However, this process is rather slow and develops over tens of seconds. Also, it has been suggested that the reduction of the hyperpolarizing IPSPs that follows tetanic stimulation could be partly due to intracellular Cl<sup>-</sup> accumulation resulting from passive flow of Cl<sup>-</sup> ions through GABA-activated Cl<sup>-</sup> channels (McCarron and Alger 1985), which has been shown to significantly decrease the IPSP driving force (Thompson and Gähwiler 1989). These results explain the reduction of IPSP amplitude but cannot explain the biphasic response or the depolarizing IPSPs when the neuron membrane potential was held more positive than the IPSP reversal potential.

Staley et al. (1995) have proposed that Cl<sup>-</sup> ions are responsible for the hyperpolarizing phase and HCO<sub>3</sub> ions are responsible for the depolarizing phase of the biphasic IPSPs in hippocampal neurons. However, in our experiments exposure of the neurons to a HCO<sub>3</sub>-free solution had no effect on the time course and amplitude of the IPSP decay phase, and we therefore concluded that the depolarizing phase of the IPSPs was not mediated by HCO<sub>3</sub> ions. Grover et al. (1993) also examined the influence of HCO<sub>3</sub> on depolarizing IPSPs in hippocampal pyramidal neurons and concluded that superfusion with HCO<sub>3</sub> did not produce shifts in the IPSP reversal potential but did reduce their amplitudes. They accounted for these effects by changes in extracellular acidification due to the lower buffering capacity of the HEPES buffer when compared with the HCO<sub>3</sub>/CO<sub>2</sub>-buffered solution. Consequently, replacement of the HCO<sub>3</sub>/CO<sub>2</sub>-buffered extracellular solution with HEPES buffer results in nonspecific changes rather than the involvement of the HCO<sub>3</sub> in the generation of biphasic and depolarizing responses.

Other investigators have studied the role of extracellular K<sup>+</sup> concentration on IPSP characteristics. It has been shown, using potassium-sensitive electrodes, that either high-frequency stimulation or application of GABA results in an increased extracellular K<sup>+</sup> concentration (Benninger et al. 1980; Heinemann and Lux 1977; Malenka and Kocsis 1982). For example, it has been demonstrated that stimulation of hippocampal slices at frequencies of 2–30 Hz results in an increase in extracellular K<sup>+</sup> concentration from 5 to 12 mM (Benninger et al. 1980). The mechanism that may contribute to the increase in external K<sup>+</sup> caused by activation of GABA<sub>A</sub> receptors includes an outward counter/cotransport of K<sup>+</sup> with Cl<sup>-</sup>/HCO<sub>3</sub> (Kaila 1994). Redistribution of K<sup>+</sup> and Cl<sup>-</sup> ions leads to a reduction in the IPSP driving force and changes in reversal potential (Barker and Ransom 1978; Thompson 1994). The contribution of elevated extracellular K<sup>+</sup> in depolarizing IPSPs has also been studied by Wong and Watkins (1982), who demonstrated that an increase in extracellular K<sup>+</sup> could increase the amplitude of the depolarizing GABA response. We also examined the influence of extracellular K<sup>+</sup> concentration on the IPSPs recorded from rNST neurons. Elevation of the extracellular K<sup>+</sup> concentration suppressed the reduction of the IPSP amplitude during tetanic stimulation and changed the IPSP reversal potential. We have therefore concluded that tetanic...
stabilization of GABAergic neurons in the rNST results in an elevation of extracellular K⁺ concentration and accumulation of intracellular Cl⁻, which changes the IPSP reversal potential. This redistribution of Cl⁻ and K⁺ produces a decay of the IPSP amplitude and as a consequence results in biphasic or depolarizing IPSPs.

**Functional significance**

It is becoming clear that afferent information arriving at the rNST is not only transmitted rostrally but results in the initiation of inhibitory activity in GABAergic second-order neurons. The results of the present study indicate that afferent input at frequencies reported in the literature would result in complex inhibitory activity. In fact the tetanic stimulation we employed results in short-term plastic changes in the inhibitory synapses of the rNST. Moreover, the short-term changes in the IPSPs are influences by the frequency of the tetanic stimulation. Thus the frequency of the afferent input to the rNST would potentially result in different inhibitory activity. In addition, as discussed above, the high-frequency afferent input is capable of suppressing spontaneous activity resulting in modulation of any chronic inhibitory activity present in rNST.

The rNST processes gustatory and somatosensory information and distributes this information to both rostral brain areas as well as to premotor brain stem nuclei. Thus sensory information processed by the rNST not only results in taste perception but also leads to changes in facial expression, various oral motor reflexes, and alterations in salivary secretion. These complex functions require control of timing, and in other systems inhibitory interneurons have been shown to synchronize the discharge of large populations of neurons (Singer 1996). It is possible therefore that these short-term changes in inhibitory synaptic activity are a key element in the organization of complex oral reflex activity.

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