Neurotransmitter and Neuromodulator Activity in the Gustatory Zone of the Nucleus Tractus Solitarius

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Abstract

The rostral nucleus of the solitary tract (rNST) is the first central relay in the gustatory pathway. While previous investigations have provided a wealth of information on the pattern of central terminations of gustatory afferent fibers, the morphology of synaptic connections of rNST neurons and responses of second order neurons to taste stimuli applied to the tongue, little is known regarding the neurophysiological characteristics of synaptic transmission in rNST. We have used an *in vitro* brain slice preparation of the rNST to study the intrinsic biophysical properties, neuropharmacology and synaptic responses of rNST neurons. These experiments have revealed that rNST neurons respond to the excitatory amino acid neurotransmitter glutamate, as well as the inhibitory amino acid neurotransmitter γ amino butyric acid (GABA). By use of glutamate receptor agonists and antagonists we have shown that rNST neurons have AMPA/kainate and NMDA ionotropic glutamate receptors, as well as matabotropic glutamate receptors. In addition, rNST neurons respond to both $GABA_A$ and $GABA_B$ receptor agonists. The nature of the transmission at the synapse between primary afferent fibers and second order neurons in rNST has been examined by electrical stimulation of the solitary tract to elicit post-synaptic potentials (PSP). Three types of monosynaptic PSP result from stimulation of the solitary tract: excitatory post-synaptic potentials, inhibitory post-synaptic potentials, and a complex mixture of excitatory and inhibitory potentials. These new discoveries provide details about synaptic transmission in rNST and thereby clarify the underlying mechanism by which gustatory information is processed. **Chem. Senses 21: 377–385, 1996.**

The rostral zone of the nucleus of the solitary tract (rNST) is the site of the first central synapse for gustatory afferent fibers that innervate taste buds of the oral cavity. Gustatory fibers in the facial (VII) and glossopharyngeal (IX) nerves enter the brainstem, and cross the trigeminal tract and nucleus to form the solitary tract (ST) (Hamilton and Norgren, 1984; Bradley *et al.*, 1985; Housley *et al.*, 1987; Hanamori and Smith, 1989). The facial nerve projection terminates in the most rostral extent of rNST and the glossopharyngeal nerve terminates caudally to the facial nerve. Using neural tracing techniques the gustatory input to the rNST has been defined in some detail. The chorda tympani branch of the facial nerve innervating taste buds on the anterior tongue terminates medial to the solitary tract in a region called the rostral central subdivision (Whitehead and Frank, 1983; Whitehead, 1988). This subdivision is also the site of termination of the lingual branch of the glossopharyngeal nerve which innervates taste buds on the posterior tongue. The projection zones of these two taste nerves are not fully segregated since their distributions overlap within the rNST (Hamilton and Norgren, 1984).

The neurons of rNST do not have a homogeneous morpho-

logy. Recent analysis of Golgi stained material of the gustatory zone of the rat, hamster and sheep rNST has shown that it contains at least three neuron types. Multipolar (stellate) neurons have a triangular or polygonal shaped soma and three to five primary dendrites; elongate (fusiform) neurons are characterized by an elongated soma and two thick primary dendrites that exit the cell body at opposite poles; ovoid neurons have small soma diameters, and two to four primary dendrites that are thin and sparsely branched (Davis and Jang, 1988; Lasiter and Kachele, 1988a, b; Whitehead, 1988; Mistretta and Labyak, 1994). Recently, a more extensive classification has been proposed based on computer reconstructions of biocytin filled rNST neurons that respond to gustatory stimulation of the tongue (Renehan *et al.*, 1994).

Some indication that these different neuron types have different functions has been derived from studies of their connections. Multipolar and elongate neurons have been shown to project rostrally to the pontine gustatory relay (Lasiter and Kachele, 1988a, b; Whitehead, 1990). These projection neurons are generally contained in the central subdivision of rNST. Multipolar neurons in the ventral part of rNST project to the reticular formation and the motor nuclei of cranial nerves V, VII, IX, X and XII (Norgren, 1978; Travers, 1988; Beckman and Whitehead, 1991). Based on these connections, the rostrally directed neurons are believed to be important in processing and relaying gustatory information, while the caudally connected neurons are presumably involved in the reflex control of salivation and ingestive behavior (Beckman and Whitehead, 1991). The ovoid neurons are hypothesized to be interneurons because they are relatively small, and in labeling studies they do not project to the pontine taste relay, the caudal NTS or the medullary reticular formation (Lasiter and Kachele, 1988a, b; Davis, 1993).

Most of the information on the functional characteristics of neurons in the rNST has come from extracellular recordings. In these experiments the rNST is probed with an electrode until a single neuron has been isolated. Taste stimuli are then flowed over the tongue, and the response of the rNST neuron to the taste stimuli is recorded and subsequently analysed. Using this classic neurophysiological approach much fundamental information has been obtained on the response properties of rNST neurons.

Most investigators report that tongue stimulation with taste stimuli results in excitatory responses producing an increased frequency of neural activity in rNST neurons (Travers and Smith, 1979; Bradley and Mistretta, 1980; Ogawa *et al.*, 1984; Renehan *et al.*, 1996). The relative magnitudes of excitatory response to different taste modalities vary in different rNST locations (Halpern, 1965; McPheeters *et al.*, 1990). When compared to responses of peripheral taste fibers, second order rNST neurons respond with a higher frequency and have a greater level of spontaneous activity (Doetsch and Erickson, 1970). The pattern of neural discharge of second order rNST neurons is different from peripheral gustatory fibers (Doetsch and Erickson, 1970), and second order gustatory neurons are more broadly responsive to taste stimuli than peripheral fibers (Travers and Smith, 1979). Moreover, there is extensive convergence of first order taste fibers onto second order neurons and the convergence apparently maximizes responsiveness to some chemicals relative to others (Vogt and Mistretta, 1990). This extensive convergence is ordered so that receptive fields of rNST neurons consist of contiguous clusters of fungiform papillae, all of which are connected to the same second order neuron (Vogt and Mistretta, 1990). Combined functional and structural studies suggest that multipolar neurons might receive a major portion of the converging input (Mistretta and Labyak, 1994).

Despite the considerable knowledge derived from these extracellular studies, little is known of the underlying neural mechanisms involved in sensory processing by the rNST because conclusions about how the gustatory nucleus encodes sensory information have been made without knowledge of the underlying neural circuits. Intracellular techniques are required to answer fundamental questions regarding neural mechanisms that transform, distribute and integrate sensory information at the first relay in the taste pathway. Because it is difficult to make stable intracellular recordings from rNST neurons *in vivo*, we have used a brain slice preparation of the rNST to investigate the biophysical, neuropharmacological and correlative morphological data on rNST neurons (Bradley and Sweazey, 1990, 1992; King *et al.*, 1993; Wang and Bradley, 1993, 1996; King and Bradley, 1994; Tell and Bradley, 1994) and, based on these findings and published morphological data, have begun to provide details of the neural circuits involved in processing taste information that travels to the rNST in the gustatory nerves. In this review, we plan to summarize our findings regarding neurotransmission in rNST. We will first discuss the effects of inhibitory and excitatory transmitters on rNST neurons and then provide information on the primary afferent synapse in the central taste pathway.

All of the work reported here was accomplished with a brain slice preparation of the rat rNST. Details of the

preparation of these slices have been given elsewhere (Bradley and Sweazey, 1992). In our initial study we used sharp electrodes to record from the rNST neurons, but even with the very stable recording conditions provided by a brain slice it proved difficult to obtain and hold neurons (Bradley and Sweazey, 1990). These problems were overcome by using the whole cell configuration of the patch clamp technique to make intracellular recordings in brain slices as described by Blanton *et al.* (1989). Not only were we able perform voltage clamp analysis of membrane currents and to reliably obtain intracellular recordings from rNST neurons, but we could also easily fill the neurons with biocytin to characterize their morphology. Moreover, because we could control the extracellular environment, we could superfuse neurotransmitter agonists and antagonists over the slices at known concentrations. The ability to control both the intracellular and extracellular environment permitted us to make conclusions regarding the ionic mechanisms involved in neurotransmission. All the data reported here were obtained in the current clamp mode by injecting current to modify the neuron's membrane potential.

To study the afferent synapse, synaptic potentials were elicited by electrically stimulating the solitary tract (ST). The electrical stimulation results in release of neurotransmitters from the presynaptic terminal which then binds to post-synaptic receptors to initiate post-synaptic potentials in the rNST neurons. The identity of the neurotransmitter was examined by using specific antagonists which, by blocking the post-synaptic receptors, alter the synaptic potentials.

Finally, in many of the experiments we report here, the intracellular label, biocytin was included in the recording pipette contents so that the morphology of the neuron could be subsequently determined (Horikawa and Armstrong, 1988). This powerful technique permits direct correlations to be made between the structure and function of rNST neurons in brain slices.

Influence of GABA, substance P and glutamate on rNST neurons

In a study of the passive membrane properties and repetitive firing characteristics of rNST neurons we found that membrane hyperpolarization has an important influence on intrinsic firing properties of rNST neurons (Bradley and Sweazey, 1992). For example, we found that if some neurons were first hyperpolarized and then depolarized, a long delay occurred in the initiation of spikes by the depolarization. Thus, membrane hyperpolarization could inhibit or change the spike discharge pattern of rNST neurons. *In vivo* hyperpolarization results from inhibitory input to a neuron, and there is a growing body of evidence indicating that inhibition may have a significant role in sensory processing in the rNST. For example, spontaneous activity of some rNST neurons is reduced by some stimuli (Travers and Smith, 1979; Ogawa *et al.*, 1984), and inhibitory interaction occurs in the region of the rNST receiving input from both the chorda tympani and glossopharyngeal nerves (Halpern and Nelson, 1965; Sweazey and Smith, 1987). Because GABA is the major inhibitory neurotransmitter in the CNS we decided to examine its effects on rNST neurons in brain slices (Wang and Bradley, 1993).

A concentration-dependent reduction in input resistance was observed during superfusion of saline containing GABA over the brain slices while recording from a neuron. Of all the neurons tested, 68% responded to GABA, the rest being unaffected. The change in input resistance was often accompanied by membrane hyperpolarization (Figure 1). By injecting current into the neuron to change the membrane potential it was possible to reverse the membrane hyperpolarization caused by GABA into a depolarizing potential.

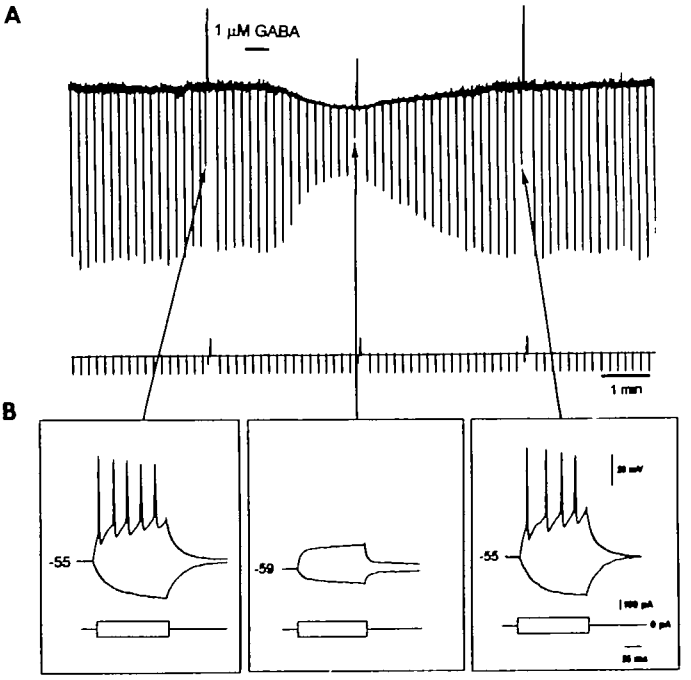

Figure 1 **(A)** Intracellular recordings from a rNST neuron showing the membrane response (top) to −100 pA constant current injections (bottom). When the superfusate contained GABA (bar) the membrane was hyperpolarized and the input resistance as indicated by the magnitude of the membrane hyperpolarizations was reduced **(B)** In the three panels the response of this neuron to a hyperpolarizing and depolarizing current pulse are shown at an increased trace speed.

This reversal potential of the GABA effect had a mean value of −60 mV.

GABA binds to two distinct types of receptors that differ in their pharmacology, biochemistry and electrophysiological characteristics (Bowery *et al.*, 1987). $GABA_A$ receptors are associated with membrane Cl^- channels, whereas $GABA_B$ receptors are associated with K^+ channels. To determine the types of GABA receptors present on rNST neurons we used agonists specific for each type of receptor. Mucimol is an agonist for $GABA_A$ receptors and baclofen is a $GABA_B$ receptor agonist. Superfusion of the slices with either the $GABA_A$ agonist, muscimol, or the $GABA_B$ agonist, baclofen, caused a decrease in input resistance accompanied by membrane hyperpolarization. Use of specific antagonists of the GABA receptors also indicates receptor specificity. The $GABA_A$ antagonist bicuculline either totally or partially blocked the neural response to GABA and blocked the response to muscimol, but did not antagonize responses to baclofen. Superfusion of the $GABA_B$ antagonist phaclofen depressed the membrane responses to GABA. The use of $GABA_A$ and $GABA_B$ agonists and antagonists demonstrates that some neurons in rNST have both $GABA_A$ and $GABA_B$ receptors. The results of this study were the first direct demonstration that most rNST neurons respond to GABA and indicates that inhibition in rNST is mediated by GABA.

In addition to inhibition, several anatomical studies have indicated that the rNST is influenced by descending input. Combined anterograde and retrograde tracing studies have shown that several forebrain nuclei project to the rNST (van der Kooy *et al.*, 1984; Yasui *et al.*, 1991). These descending projections include the medial and lateral prefrontal cortex, bed nucleus of the stria terminalis, central nucleus of the amygdala, hypothalamic paraventricular nucleus, perifornical nucleus, arcuate nucleus and posterolateral nucleus of the hypothalamus. Thus, the rNST receives an extensive descending input. Because there is immunocytochemical evidence that descending inputs to rNST contain SP (Kalia *et al.*, 1984), we examined the effects of SP on rNST neurons.

Superfusion of SP over brain slices had a marked effect on the biophysical properties of rNST neurons (King *et al.*, 1993). SP transiently depolarized 65% of rNST neurons in a dose-dependent manner. Submicromolar concentrations of SP had potent excitatory effects, and the half maximal response occurred at 0.6 μM. The depolarizing effect of SP was accompanied by an increase in input resistance in 81% of the responsive neurons (Figure 2). Although SP might be acting at the primary afferent synapse to rNST, it might also be derived from other brainstem and forebrain areas known

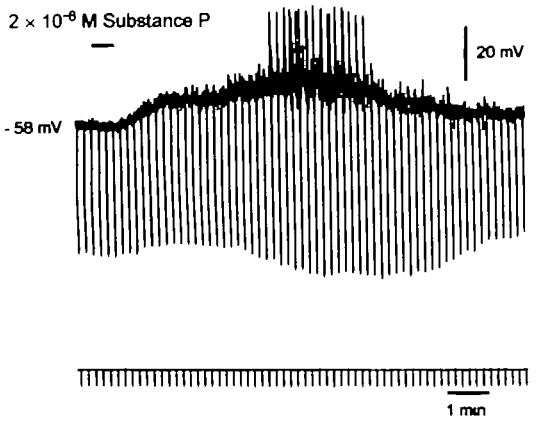

Figure 2 Intracellular recordings from a rNST neuron showing the membrane response (top) to −100 pA constant current injections (bottom). When the superfusate contained Substance P (bar) the membrane was depolarized, action potentials were generated and the input resistance as indicated by the magnitude of the membrane hyperpolarizations was increased.

to contain SP that project to rNST. Thus, we have suggested that SP is involved in the descending input to the rNST from more rostral brain areas.

Recently, other investigators have used a slice preparation of the hamster rNST to study the effects of GABA and SP on rNST neurons (Liu *et al.*, 1991, 1992, 1993). As in the rat, GABA hyperpolarized the cell membrane and decreased input resistance, and these effects were blocked by bicuculline. Application of SP depolarized hamster rNST neurons. Thus, GABA and SP have similar membrane effects in both the rat and hamster rNST.

A number of investigators have demonstrated that glutamate is the putative neurotransmitter involved in neurotransmission at the primary afferent synapse in the caudal NST (Meeley *et al.*, 1989; Andresen and Yang, 1990, 1994; Drewe *et al.*, 1990; Glaum and Miller, 1992; Felder and Mifflin, 1994). Moreover, glutamate is the major excitatory neurotransmitter in the CNS. We therefore examined the responses of rNST neurons to glutamate (Shu and Bradley, 1994). Glutamate post-synaptic ionotrophic receptors have been extensively characterized and are divided into AMPA (α-amino-3-hydroxy-5-methyl-isoxazole-4-proprionic acid) and NMDA (N-methyl-D-aspartate) receptors named according to their characteristic agonists.

AMPA excited 82% of rNST neurons resulting in membrane depolarization and initiating spike activity. Membrane input resistance was reduced by application of AMPA in a dose-dependent manner over a concentration range of 0.25–10 μM and reached a plateau at 5 μM (Figure 3). The AMPA reversal potential estimated from the intersection

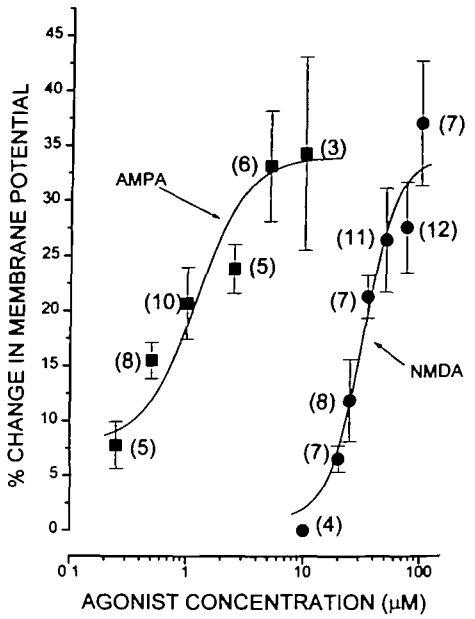

Figure 3 Mean response concentration function of rNST neurons to the application of AMPA or NMDA. The values in parentheses represents the number of measurements for each point.

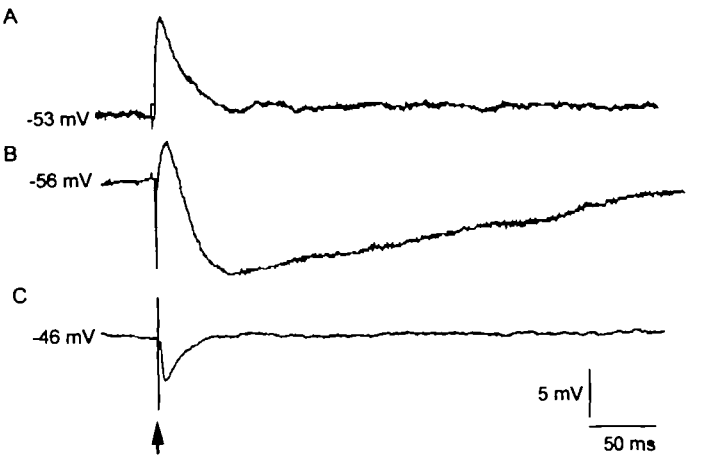

Figure 4 Typical post-synaptic potentials recorded from rNST neurons to electrical stimulation of the solitary tract (arrow). **(A)** An excitatory post synaptic potential; **(B)** A mixed excitatory and inhibitory post-synaptic potential; **(C)** an inhibitory post-synaptic potential.

of current-voltage curves under control and experimental conditions was −4.4 ± 6.1 mV.

Application of NMDA elicited responses in all rNST neurons tested. NMDA depolarized the neurons and initiated action potentials. The depolarization was dose-dependent over a concentration range of 10–100 μM (Figure 3). For most of the neurons tested, NMDA produced a decrease in input resistance, but in 10% of the neurons, NMDA caused an increase in input resistance. The reversal potential for NMDA was −8.2 ± 1.5 mV. This value was almost the same as the AMPA reversal potential indicating that similar ionic mechanisms were involved.

Synaptic potentials of rNST neurons

Because the synaptic transmitters were superfused over the slices we were not able to determine whether these excitatory and inhibitory neurotransmitters were involved in synaptic transmission between primary afferent fibers and second-order rNST neurons. In fact, little is known about the properties of transmission at this synapse because it is difficult to obtain intracellular recordings from second-order rNST neurons *in vivo*. We used the brain slice preparation of the rat medulla so that synaptic responses of rNST neurons to stimulation of the ST could be recorded intracellularly. In addition, we have examined the identity of the neurotransmitter(s) involved in excitatory and inhibitory synaptic potentials of second order rNST neurons (Wang and Bradley, 1995).

Spontaneous synaptic events were rarely observed in rNST neurons at resting membrane potentials. When spontaneous synaptic potentials were encountered, they were mostly in depolarizing, but hyperpolarizing potentials were sometimes present. A post-synaptic potential (PSP) was always evoked by stimulation of the ST, and consisted of either a depolarizing excitatory post-synaptic potential (EPSP), a hyperpolarizing inhibitory post-synaptic potential (IPSP) or a mixed EPSP/IPSP response (Figure 4). The most frequently encountered PSPs were depolarizing which were elicited from 65% of the neurons. The amplitude of these depolarizing PSPs increased with increasing stimulus strength and once above threshold, fired an action potential. For most neurons the amplitude of the depolarizing potentials decreased with decreasing membrane potential except at membrane potentials between −50 and −60 mV when there was an increase. This non-linearity in the relationship between membrane potential and PSP amplitude revealed that, although the EPSPs are a major contributor to these PSPs, inhibitory potentials that reverse around −60 mV are involved. For some neurons the relationship between membrane potential and depolarizing PSP was linear with a reversal potential close to 0 mV indicating that these were pure excitatory PSPs. Pure inhibitory potentials were elicited from 22% of rNST neurons with a reversal potential of –59 mV.

Because glutamate has been shown to be involved in excitatory transmission at the afferent synapse in the caudal non-gustatory NTS, and because we have shown that rNST

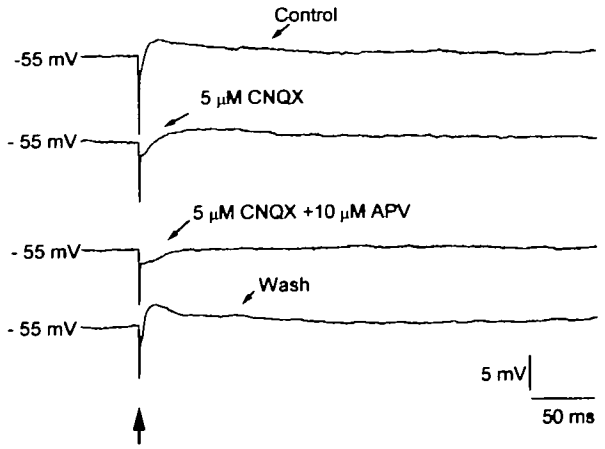

Figure 5 Average of 10 PSPs before during and after the application of CNQX and APV CNQX partially blocks the PSP and when combined with APV completely eliminates the PSP. The PSP recovers following a return to control conditions.

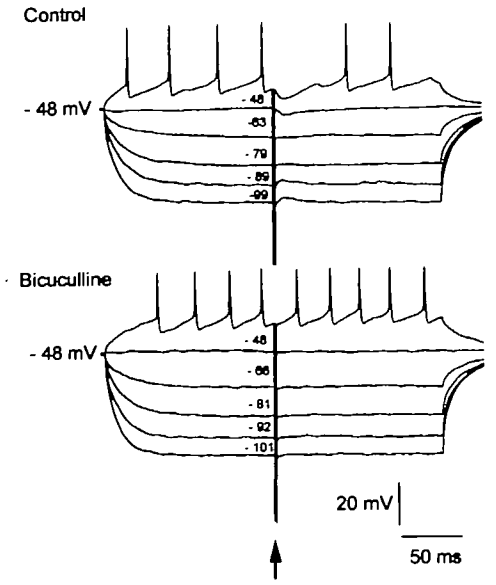

Figure 6 Post-synaptic potentials recorded from a rNST neuron. The membrane potential was changed by injecting a series of hyperpolarizing and one depolarizing current steps The solitary tract was stimulated at the arrow Numbers above each trace indicate the step voltage just prior to the solitary tract stimulation. Note in the control traces that the inhibitory PSP reversed at around −60 mV. In the lower panel the same current injections were applied after the neuron had been exposed to the $GABA_A$ receptor blocker bicuculline Note that this completely blocks the inhibitory PSPs.

neurons respond to both AMPA and NMDA, we initiated a series of experiments to determine if glutamate was responsible for excitatory transmission at the afferent synapse in rNST. We examined the effects of glutamate antagonists on EPSPs. CNQX (6,7-dinitroquinoxaline-2,3-dione) in low Mg^{2+} saline, which selectively blocks AMPA receptors, reduced the magnitude of EPSPs in rNST neurons indicating that AMPA receptors are involved in afferent transmission in rNST. APV (D-2-amino-phosphovalerate), an NMDA receptor antagonist, eliminated the CNQX resistant portion of the EPSPs, indicating that NMDA receptors are involved in afferent transmission as well (Figure 5).

We have recently obtained evidence that metabotropic receptors are involved in the afferent synapse, as well as ionotropic glutamate receptors. ACPD (trans-1-aminocyclopentane-1,3-dicarboxylic acid), a specific agonist of the metabotropic glutamate receptor, caused a concentration-dependent depression of EPSPs and IPSPs which suggested that ACPD receptors are also involved in synaptic transmission in rNST.

Bicuculline, the $GABA_A$ antagonist, either blocked the IPSP component of EPSP/IPSP complexes or completely eliminated IPSPs (Figure 6). The $GABA_B$ receptor antagonist phaclofen was also effective in reducing the amplitude of IPSPs in some rNST neurons.

These results are the first description of the characteristics of the synapse between primary afferent fibers and second-order neurons of rNST. While EPSPs predominate, IPSPs have a considerable role at the afferent synapse. For the first time glutamate has been identified as an excitatory and GABA as an inhibitory neurotransmitter at this synapse.

Implications

They key to understanding how gustatory information is processed and distributed by the rNST lies in a thorough knowledge of the underlying circuit and synaptic interactions that take place at this first relay in the central taste pathways. Until very recently, the sum of our knowledge of central taste circuits was at the level of a point to point description of the central taste pathways. In other sensory systems there is a basic understanding of the neural circuits involved in sensory processing. For example, there is a wealth of detail on types of neurons, interconnections and neuropharmacology of the first central relay in the olfactory system—the olfactory bulb (Mori, 1987; Scott and Harrison, 1987; Shepherd and Greer, 1990; Greer, 1991; Kauer and Cinelli, 1993; Scott *et al.*, 1993). This basic knowledge is fundamental to understanding how the olfactory bulb codes olfactory information. In contrast, the same level of understanding is lacking for the first relay in the taste system yet conclusions regarding how the gustatory nucleus encodes sensory information have been made without knowledge of the underlying neural circuits.

Despite the missing information on circuits of rNST, the

brainstem gustatory relay has often been treated as a relatively simple synaptic processing nucleus and little attention has been focused on some vary basic considerations. For example, neurons in the rNST are often referred to as 'taste neurons' or 'salt best neurons' without any direct knowledge of what these neurons actually do. Is a taste neuron merely a neuron that responds when taste stimuli are place on the tongue or is a taste neuron a neuron that relays information leading to a perceptual response rather than initiating secretory or motor reflexes? Or are all of these examples taste neurons? Until such basic questions are addressed, understanding what the rNST does will not be possible.

To study taste coding it is essential to realize that the rNST is, in fact, quite complex. Morphological studies by several investigators have demonstrated that the rNST contains at least three neuron types, that the synapses between neurons are complex, and that rNST neurons project to a number of different rostral and caudal brain areas. Moreover, a major proportion of the cells in the rNST are interneurons and the rNST receives a number of descending inputs from rostral brain areas. This morphological evidence implies that the rNST is a rather complex nucleus and is at least as complex as the olfactory bulb. The recent work from our laboratory reviewed here also demonstrates complexity in rNST synaptic activity, involving both excitatory and inhibitory synaptic potentials mediated by several neurotransmitters acting at a number of neurotransmitter receptors. Moreover, future research will no doubt add additional details to the complexity of this nucleus as much remains to be learned regarding the neuropharmacology and synaptic characteristics of rNST neurons and little is known regarding the synaptic interactions between primary afferent taste fibers of the VIIth and IXth nerves, and second order rNST neurons. The role of the rNST interneurons remains completely unknown. Much work, therefore, remains to be accomplished regarding the basic neural substrate of gustatory processing. The *in vitro* brain slice preparation that has been extremely useful in other parts of the central nervous system is now proving to be just as useful in revealing details of neurotransmission in the medullary taste relay.

ACKNOWLEDGEMENTS

This work was supported by National Institute of Deafness and Other Communications Disorders Grant DC-00288 to R.M. Bradley.

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