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Effects of GABA on Acutely Isolated Neurons from the Gustatory Zone of the Rat Nucleus of the Solitary Tract

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Abstract

Responses of acutely isolated neurons from the rostral nucleus of the solitary tract (rNST) to GABA receptor agonists and antagonists were investigated using whole-cell recording in current clamp mode. The isolated neurons retain their morphology and can be divided into multipolar, elongate and ovoid cell types. Most rNST neurons (97%), including all three cell types, respond to GABA with membrane hyperpolarization and a reduction in input resistance. The $GABA_A$ receptor agonist muscimol reduces neuronal input resistance in a concentration-dependent manner, whereas the $GABA_B$ receptor agonist baclofen had no effect on any of the neurons tested. The GABA and muscimol reversal potentials were both found to be −75 mV. Both the GABA competitive antagonist picrotoxin and the $GABA_A$ receptor antagonist bicuculline block the effect of GABA in a concentration-dependent manner. These results suggest that GABA activates all neurons in the rNST and that inhibitory synaptic activity is important in brainstem processing of gustatory and somatosensory information.

Introduction

The rostral nucleus of the solitary tract (rNST) is the first central relay responsible for processing taste information. In recent work from this laboratory we have shown that inhibition, mediated by GABA, is an important component of synaptic processing in rNST (Wang and Bradley, 1995; Grabauskas and Bradley, 1996, 1998; Bradley and Grabauskas, 1998). Our approach to study rNST inhibition has been to initiate postsynaptic potentials by electrical stimulation of the solitary tract and nucleus in a brain slice preparation. While this technique has provided valuable insight into rNST inhibitory activity, synaptic potentials recorded from neurons with intact synaptic inputs are complex mixtures of excitatory and inhibitory potentials. Furthermore, in this preparation it is not easy to determine the morphological identity of neurons responding to GABA. Because acutely isolated rNST neurons retain their morphology and biophysical characteristics (Du and Bradley, 1996) and are isolated from synaptic inputs, it is possible to examine the responses of rNST neurons to GABA and correlate the responses with cell morphology.

In this report we have used acutely dissociated rNST neurons and whole-cell recording techniques to examine the effects of GABA on identified neurons in rNST. The results demonstrate that GABA inhibits all morphological types of neurons in rNST, indicating that inhibition has widespread influences on sensory processing by the rNST.

Materials and methods

Neurons were freshly isolated from the NST of rats aged 8–24 days in an area rostral to the point where the medial border of the NST separates from the lateral edge of the fourth ventricle. This region is innervated by gustatory nerves (Hamilton and Norgren, 1984) and therefore contains principal and interneurons involved in processing gustatory information.

Rats were anesthetized with sodium pentobarbital (50 mg/kg) and decapitated. The brain was rapidly removed and placed in ice-cold HEPES buffer containing (in mM) 124 NaCl, 5 KCl, 5 $MgCl_2$, 10 Na-succinate, 15 dextrose, 15 HEPES and 2 $CaCl_2$ gassed with O_2. The pH was adjusted to 7.4 with 1 M NaOH. Horizontal 300-μm-thick brainstem slices were cut on a Vibratome and placed in a holding chamber filled with the HEPES buffer at room temperature.

After incubating the slices for 1.0–1.5 h, the left and right rNST identified by transillumination from below were microdissected from the slices, transferred to the HEPES buffer containing 0.5% protease (Sigma type 23) warmed to 37°C and gently bubbled with O_2 for 30 min. The rNST pieces were removed from the protease and placed in 100 ml

of oxygenated HEPES buffer to stop the enzymatic reaction. The rNST was then triturated with a series of progressively smaller diameter, fire-polished Pasteur pipettes to produce a suspension of dissociated neurons which were placed in a 35 mm diameter plastic Petri dish held in a block of plastic on the stage of an inverted microscope. Once the cells adhered to the bottom of the Petri dish, they were superfused with oxygenated HEPES buffer.

Whole-cell, current clamp recordings were made using an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA). Patch electrodes pulled from 1.5 mm OD borosilicate filament glass were filled with a solution containing (in mM) 130 potassium gluconate, 10 HEPES, 10 EGTA, 1 $MgCl_2$, 1 $CaCl_2$ and 2 ATP. The pipette solution was adjusted to pH 7.2 with KOH and had an osmolarity of 275–292 mOsm. The final electrode resistance was 5–8 MΩ. 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. Criteria for a successful recording included a minimum of 10 min recording time with a stable resting membrane potential of >–40 mV, action potential amplitude of >50 mV, a mean duration measured at half amplitude of <6 ms and neuron input resistance of >120 MΩ.

A continuous flow of HEPES buffer was maintained over the cells. Electrodes were manipulated under visual control. Drugs diluted in the HEPES buffer were applied directly to the neurons through a four-channel pipette positioned close to the cell soma connected to a Picrospritzer (General Valve, Fairfield, NJ). The stimulus pipette could also be repositioned to stimulate the cell dendrites. Because the HEPES buffer was constantly flowing, the drugs were emitted as a plume that was rapidly washed away by the flowing buffer. Drugs were applied for 30 s and the neuron was then superfused with control buffer for at least 1 min before applying another concentration of a drug. Drugs applied were GABA, bicuculline, muscimol, picrotoxin (all supplied by Sigma, St Louis) and baclofen (Research Biochemicals International, Natick, MA). Final concentrations were made immediately prior to the recordings.

All data were acquired and analyzed using pCLAMP (Axon Instruments). Data was also recorded on videotape using a pulse code modulated adapter (Vetter, Rebersberg, PA) for later analysis and preparation of figures. Changes in neuron input resistance were monitored by injecting negative constant current pulses (100 ms, 0.1 nA) into the neurons at a frequency of 0.15 Hz, the resultant changes in membrane potential being recorded continuously on a pen recorder at slow speed. Statistical analysis and data plotting was performed using the Microcal Origin software program. The numerical value are given as mean ± SE.

Results

Biophysical properties of the isolated neurons

The results are based on recordings from 92 neurons dissociated from 69 rats. Neurons had a spike overshoot of at least 10 mV. Resting membrane potentials ranged from –40 to –89 mV, with a mean of –55 ± 1mV. Action potential amplitudes were between 51 and 108 mV (mean 71 ± 1 mV), with a mean duration measured at half amplitude of 3.4 ± 0.1 ms. Input resistance ranged from 113 to 697 MΩ and averaged 392 ± 15 MΩ. Membrane time constants ranged from 4 to 62 ms and averaged 27.2 ± 1.3. The dissociated neurons retained their morphology and could be classified as multipolar, elongate and ovoid neurons as described previously (King and Bradley, 1994; Du and Bradley, 1996).

Responses to GABA, muscimol and baclofen

Application of GABA and the $GABA_A$ receptor agonist muscimol to the cell soma resulted in a reduction in input resistance and membrane hyperpolarization. Seventy-four of 76 neurons (97%) responded to GABA and 27/28 neurons responded to muscimol (96%). The electrophysiological responses to application of GABA and muscimol had a rapid onset and returned to control levels immediately after the termination of the drug application. The $GABA_B$ receptor agonist baclofen was tested on 11 neurons and found to have no effect on either input resistance or membrane potential when applied to either the soma or dendrites of the isolated rNST neuron (Figure 1).

An array of four glass pipettes containing four drug concentrations was used to construct dose–response curves and determine Hill coefficients for both GABA and muscimol. Eight different concentrations of GABA (1×10^{-6}–1×10^{-3} M) and muscimol (1×10^{-7}–3.2×10^{-4} M) were applied to different groups of neurons and revealed a dose-dependent reduction in input resistance. Dose–response curves were fitted using the Hill equation,

$$R = R_{max}\,(C^n/(K_{50} + C^n))$$

where R is the concentration-dependent change in membrane resistance, R_{max} the maximum response, C the drug concentration, K_{50} the agonist concentration that produces a half-maximal response and n the Hill coefficient (Figure 2). The muscimol dose–response curve is shifted to the left of the GABA dose–response curve, indicating that muscimol is effective at lower concentrations than GABA. The EC_{50} for GABA was 5×10^{-5} M, with a Hill coefficient of 1.6. The EC_{50} for muscimol was 8.3×10^{-6} M, with a Hill coefficient of 0.8.

The GABA and muscimol reversal potentials were found by applying GABA and muscimol at different holding potentials. The mean GABA reversal potential was –75 ± 3

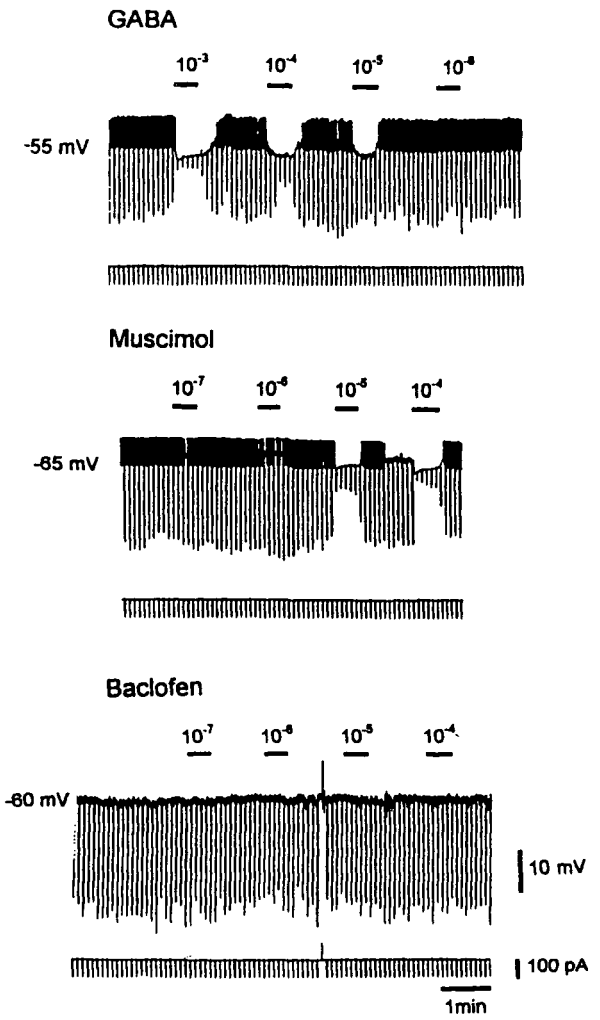

Figure 1 Membrane responses (upper trace) of three rNST neurons to constant current hyperpolarizing current pulses (lower trace) during application of GABA, the $GABA_A$ receptor agonist muscimol and the $GABA_B$ receptor agonist baclofen (horizontal bars). Between applications the neurons were exposed to control saline. The neurons respond to GABA and muscimol with membrane hyperpolarization and a reduction in input resistance in a concentration dependent manner. Baclofen at all concentrations had no effect on the membrane potential or input resistance of the isolated rNST neuron.

mV (n = 10) and the mean muscimol reversal potential was -75 ± 3 (n = 6).

Because we were able to separate the neurons by morphological characteristics (King and Bradley, 1994; Du and Bradley, 1996) we were able to compare GABA dose–response curves for multipolar and ovoid neurons. The number of elongate neurons in our sample was too small to construct dose–response curves. As can be seen in Figure 3, the dose–response curves for multipolar and ovoid neurons are essentially the same. The EC_{50} for multipolar neurons was 4×10^{-5} M, with a Hill coefficient of 1.5. The EC_{50} for ovoid neurons was 6×10^{-5} M, with a Hill coefficient of 2.3.

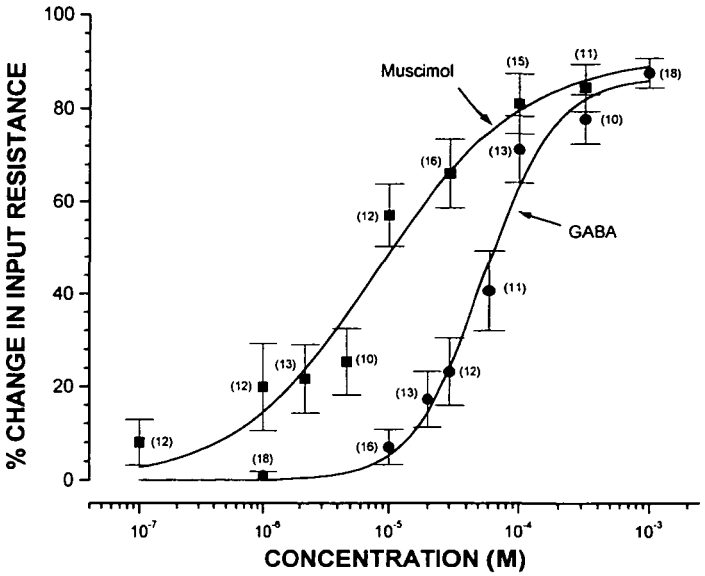

Figure 2 Concentration–response curves of the percent change in input resistance to increasing concentrations of GABA and the $GABA_A$ receptor agonist muscimol. Numbers of measurements at each concentration are shown in parentheses. The change in input resistance was measured for several different groups of neurons. The curves were fitted using the Hill equation (see text).

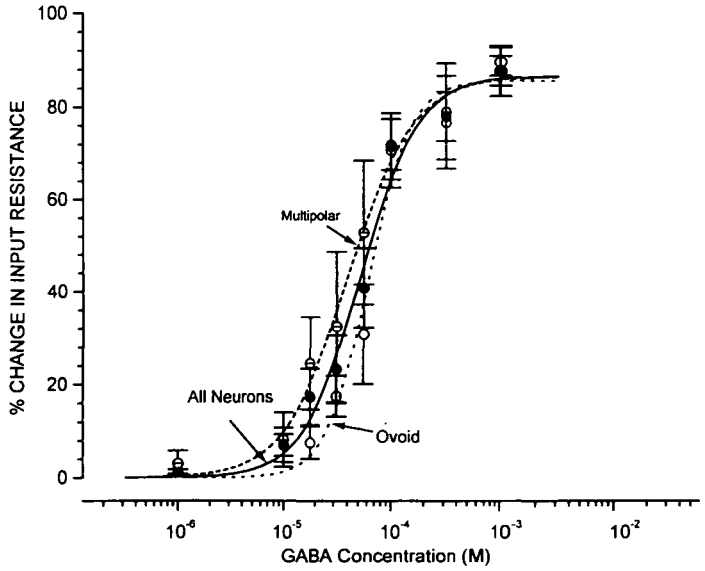

Figure 3 Concentration–response curves of the percent change in input resistance to increasing concentrations of GABA recorded in a series of rNST multipolar and ovoid neurons. The change in input resistance was measured for several different groups of neurons. The curves were fitted using the Hill equation (see text).

Membrane responses to GABA receptor antagonists

We examined the antagonist pharmacology of the GABA-evoked responses by applying GABA at a fixed concentration together with different concentrations of GABA antagonists. Bicuculline, a $GABA_A$ receptor antagonist, and

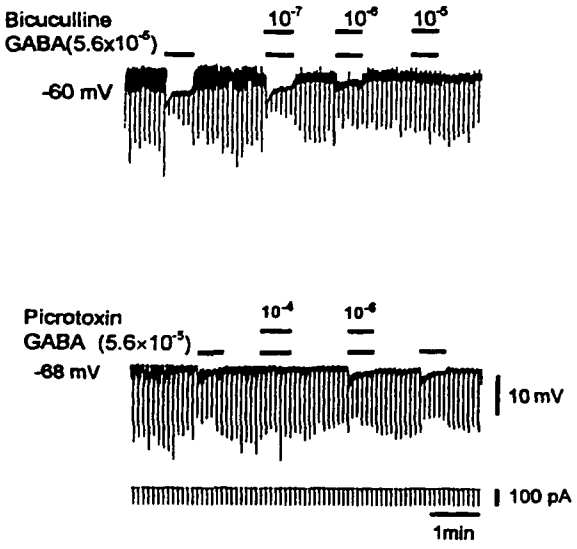

Figure 4 Membrane responses of two rNST neurons to application of different concentrations of the $GABA_A$ receptor antagonist bicuculline and the GABA competitive antagonist picrotoxin (horizontal bars) during application of GABA (5.6×10^{-5} M).

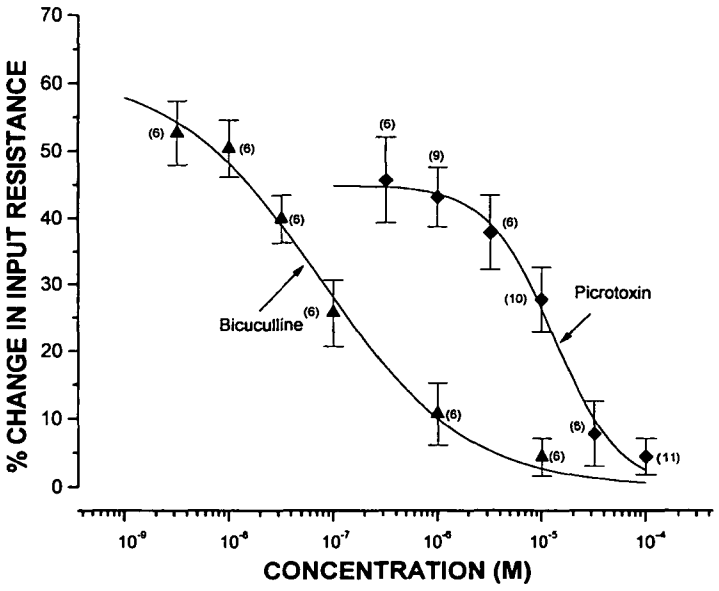

Figure 5 Concentration–inhibition relationships for the $GABA_A$ receptor antagonist bicuculline and the GABA competitive antagonist picrotoxin on 5.6×10^{-5} M GABA. Numbers of measurements at each concentration are shown in parentheses. The change in input resistance was measured for several different groups of neurons. The curves were fitted using the Hill equation (see text).

picrotoxin, a GABA competitive antagonist, blocked the GABA (5.6×10^{-5} M) responses in a concentration-dependent manner (Figure 4). Membrane potential changes were completely blocked by 1×10^{-5} M bicuculline and 1×10^{-4} M picrotoxin. Lower concentrations of bicuculline and picrotoxin partially antagonized the GABA response (Figure 5). Because the concentration–inhibition curve for bicuculline was shifted to the left of the picrotoxin curve, bicuculline is seen to be the more potent antagonist. The half maximum inhibitory concentration value for bicuculline was 7.5×10^{-8} M and that for picrotoxin was 1×10^{-5} M (Figure 5).

Discussion

All the neurons studied, including multipolar, elongate and ovoid neurons, responded to GABA, indicating that most types of rNST neuron express GABA receptors. Dissociated neurons derived from the caudal non-gustatory NST also responded to GABA (Nakagawa *et al.*, 1991). However, investigators studying caudal NST did not specifically mention a non-responsive population. Thus, the NST is similar to other brain areas in which GABA receptors are widely expressed (Nicoll *et al.*, 1990; Wisden *et al.*, 1992). In our previous study using brain slices we reported that only 68% of the rNST neurons responded to GABA (Wang and Bradley, 1993); Smith and Li (1998), recording from rNST cells *in vivo*, reported that 63% responded to GABA. Thus, using two very different approaches to the study of GABA sensitivity in rNST, a very similar proportion of neurons responded. In the brain slice study we concluded that the reason why all the rNST neurons did not respond to GABA was the failure of the drug to reach the neuron to produce an effect. Based on the results of the current study, it seems likely that this hypothesis is true since access is not a problem when using dissociated neurons. Moreover, the dose–response curve in the present study (1×10^{-6}–1×10^{-3} M) is shifted to the left of the dose–response curve of the brain slice study (1×10^{-4}–1×10^{-3} M), further indicating limitations on penetration of GABA to neurons in the brain slice preparation (Wang and Bradley, 1993). However, it is also possible that elimination of all the synaptic interactions during neuron isolation may be a factor in determining the effectiveness of GABA application. For example, it is now established that the rNST is under tonic GABA inhibition (Liu *et al.*, 1993; Wang and Bradley, 1993; Smith and Li, 1998) and, depending on the strength of this inhibition, addition of more GABA may be ineffective in causing additional inhibition.

Three different types of GABA receptors have been described in the CNS: $GABA_A$, $GABA_B$ and $GABA_C$. Of these three, the type of GABA receptors involved in most inhibitory synapes in the brain are activated by two distinctly different mechanisms. $GABA_A$ receptors are coupled to Cl^- channels and $GABA_B$ receptors are connected with a second messenger system and a change in the K^+ conductance of the cell membrane (Mody *et al.*, 1994). The results of the present study suggest that GABA responses in rNST are mediated by $GABA_A$ receptors since application of the $GABA_B$ agonist baclofen had no effect on

the sample of neurons tested. Nakagawa *et al.* (1991) reported a similar finding for neurons acutely dissociated from the cNST. However, in our study of inhibition in rNST using brain slices we reported that both $GABA_A$ and $GABA_B$ receptors are involved in inhibitory transmission in rNST (Wang and Bradley, 1993, 1995). The difference between the results obtained with the isolated neurons and brain slices may be due to the reduction in the length of the dendritic tree resulting from the isolation process. In particular it has been shown in hippocampal pyramidal cells that $GABA_B$ receptors are concentrated on the distal dendrites while $GABA_A$ receptors are located principally on the cell soma (Newberry and Nicoll, 1985). Thus, the loss of the distal dendrites on the rNST neurons could significantly reduce or eliminate responses to $GABA_B$ receptor agonists. While this is a possibility, it is also conceivable that the number of neurons sampled with baclofen was too small and that if we had used a greater sample size we would have recorded $GABA_B$ responses. In our earlier brain slice experiments the number of rNST neurons responding to $GABA_B$ recceptor agonists and antagonists was much smaller than those responding to $GABA_A$ receptor agonists and antagonists (Wang and Bradley, 1993). It is also possible that because of cellular dialysis during whole-cell recording the second messenger-coupled $GABA_B$ responses were eliminated, resulting in a lack of response to baclofen. It would be possible to resolve this in future experiments using a perforated patch recording technique.

It is apparent from the results of the current study that neural inhibition must play an important function in sensory processing in the rNST. However, the precise role played by inhibition in rNST is far from clear and, based on traditional extracellular studies, there are few data to indicate that inhibition plays any role at all in rNST processing of sensory information. Given the growing body of evidence that GABA can have a profound influence on the synaptic responses of second-order neurons in the rNST, it is pertinent to speculate what role inhibition might play in rNST to better understand how this nucleus processes taste and somatosensory information.

A traditional view of the role of inhibition is that it functions to eliminate unwanted activity (Singer, 1996) and there is evidence that this occurs in rNST. For example, during convergent input derived from receptive fields on the front and back of the tongue, we have shown that when the anterior tongue produces excitatory potentials the rear of the tongue can produce inhibitory potentials which sum in a complex manner at the second-order neuron (Grabauskas and Bradley, 1996). In addition, postsynaptic potentials in rNST neurons are complex mixtures of excitatory and inhibitory inputs, and the inhibitory component serves to shorten the time course of the postsynaptic potentials, thereby reducing excitability (Grabauskas and Bradley, 1996). In other sensory systems GABA inhibition has been shown to play a role in sharpening the responses of sensory neurons and this has recently been shown to occur in the rNST as well (Smith and Li, 1998).

While presumptive interneurons in rNST receive direct excitatory input from the afferent input, they have also been shown to receive inhibitory input from other inhibitory neurons—an example of recurrent inhibition (Grabauskas and Bradley, 1996). In other brain areas recurrent inhibition not only suppresses neural responses but also affects their temporal patterning (Singer, 1996). For example, inhibitory interneurons have been shown to induce oscillations that synchronize discharges of large populations of neurons. Thus, inhibitory interneurons can influence several projection cells and force them to pause in phase, raising the prominence of a particular sensory signal. Such a mechanism might be important in the rNST in synchronizing, for example, the facial expressions related to different tastes (Grill and Norgren, 1978) or the copious production of saliva (Bradley, 1991) in response to sour tastes.

Acknowledgements

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References

Bradley, R.M. (1991) *Salivary secretion*. In Getchell, T.V., Doty, R.L., Bartoshuk, L.M. and Snow, J.B.J. (eds), Smell and Taste in Health and Disease. Raven Press, New York, pp. 127–144.

Bradley, R.M. and **Grabauskas, G.** (1998) *Neural circuits for taste: excitation, inhibition and synaptic plasticity in the rostral gustatory zone of the nucleus of the solitary tract*. In Murphy, C. and Greer, C. (eds), Olfaction and Taste XII. New York Academy of Sciences, New York, in press.

Du, J. and **Bradley, R.M.** (1996) *Electrophysiological and morphological properties of neurons acutely isolated from the rostral gustatory zone of the rat nucleus of the solitary tract*. Chem. Senses, 21, 729–737.

Grabauskas, G. and **Bradley, R.M.** (1996) *Synaptic interactions due to convergent input from gustatory afferent fibers in the rostral nucleus of the solitary tract*. J. Neurophysiol., 76, 2919–2927.

Grabauskas, G. and **Bradley, R.M.** (1998) *Tetanic stimulation induces short term potentiation of inhibition in the rostral nucleus of the solitary tract*. J. Neurophysiol., 79, 595–604.

Grill, H.J. and **Norgren, R.** (1978) *The taste reactivity test. II. Mimetic responses to gustatory stimuli in chronic thalamic and chronic decerebrate rats*. Brain Res., 143, 281–297.

Hamilton, R.B. and **Norgren, R.** (1984) *Central projections of gustatory nerves in the rat*. J. Comp. Neurol., 222, 560–577.

King, M.S. and **Bradley, R.M.** (1994) *Relationship between structure and function of neurons in the rat rostral nucleus tractus solitarii*. J. Comp. Neurol., 344, 50–64.

Liu, H., Behbehani, M.M. and **Smith, D.V.** (1993) *The influence of GABA on cells in the gustatory region of the hamster solitary nucleus*. Chem. Senses, 18, 285–305.

Mody, I., De Koninck, Y., Otis, T.S. and **Soltesz, I.** (1994) *Bridging the cleft at GABA synapses in the brain*. Trends Neurosci. 17, 517–525.

Nakagawa, T., Wakamori, M., Shirasaki, T., Nakaye, T. and **Akaike, N.** (1991) *Gamma-aminobutyric acid-induced response in acutely isolated nucleus solitarii neurons of the rat*. Am. J. Physiol. Cell Physiol., 260, C745–C749.

Newberry, N.R. and **Nicoll, R.A.** (1985) *Comparison of the action of baclofen with t-aminobutyric acid on rat hippocampal pyramidal cells* in vitro. J. Physiol., 360, 161–185.

Nicoll, R.A., Malenka, R.C. and **Kauer, J.A.** (1990) *Functional comparison of neurotransmitter receptor subtypes in mammalian central nervous system*. Physiol. Rev., 70, 513–565.

Singer, W. (1996) *The changing face of inhibition*. Curr. Biol., 6, 395–397.

Smith, D.V. and **Li, C.** (1998) *Tonic GABAergic inhibition of taste-responsive neurons in the nucleus of the solitary tract*. Chem. Senses, 23, 159–169.

Wang, L. and **Bradley, R.M.** (1993) *Influence of GABA on neurons of the gustatory zone of the rat nucleus of the solitary tract*. Brain Res., 616, 144–153.

Wang, L. and **Bradley, R.M.** (1995) In vitro *study of afferent synaptic transmission in the rostral gustatory zone of the rat nucleus of the solitary tract*. Brain Res., 702, 188–198.

Wisden, W., Laurie, D.J., Monyer, H. and **Seeburg, P.H.** (1992) *The distribution of 13 GABAA receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon*. J. Neurosci., 12, 1040–1062.

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