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A depolarizing inhibitory response to GABA in brainstem auditory neurons of the chick

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Abstract

Neurons in the brainstem auditory nuclei, n. magnocellularis and n. laminaris, of the chick are contacted by terminals containing the inhibitory neurotransmitter γ-aminobutyric acid (GABA). In this report we describe the physiological response of these neurons to GABA using an in vitro slice preparation. In brainstem auditory neurons, GABA produced a depolarization of up to 20 mV and an associated decrease in input resistance. This depolarization was inhibitory; action potentials generated by orthodromic synaptic drive, antidromic stimulation and intracellular current injection were prevented by GABA application. The GABA response still occurred when synaptic transmission was prevented by perfusing the slice with a medium containing low Ca^{2+} and high Mg^{2+} concentrations. Thus, the effects of GABA were directly on the postsynaptic neuron and not via an interneuron. Whole-cell voltage clamp of neurons revealed that the reversal potential of the inward current was approximately −45 mV, suggesting that the channel responsible for this response is not selective for Cl^- or K^+. Pharmacological analyses suggest that this GABA receptor has properties distinct from those typical of either $GABA_a$ or $GABA_b$ receptors. Although a similar response was observed with the $GABA_a$ agonist, muscimol, it was not blocked by the $GABA_a$ antagonist, bicuculline. The response was not evoked by the $GABA_b$ agonist, baclofen, and was not blocked by the $GABA_b$ antagonist phaclofen. This unusual depolarizing response is not a common feature of all brainstem neurons. Neurons located in the neighboring medial vestibular nucleus show a more traditional response to GABA application. At resting potential, these neurons show a hyperpolarizing or biphasic response associated with a decrease in input resistance and inhibition of their spontaneous activity. GABA-induced responses in the medial vestibular nucleus are blocked by bicuculline. These results suggest that an unusual form of the GABA receptor is present in the brainstem auditory system of the chick. It is possible that this form of GABA receptor provides an efficient mechanism for inhibiting the relatively powerful EPSPs received by brainstem auditory neurons, or it may play a trophic role in the afferent regulation of neuronal integrity in this system.

Keywords: Nucleus magnocellularis; Nucleus laminaris; Medial vestibular nucleus; Inhibition; Brain slice; γ-Aminobutyric acid; Receptor

1. Introduction

An understanding of how the brain encodes sensory information requires knowledge of the neuropharmacology of the sensory system. One model system which has been used for examining the neural processing of acoustic information, as well as for investigating neural development [36–38], is the brainstem auditory system of the chick. This has been a useful system because it is relatively simple; the projection patterns of afferents are relatively homogeneous and each neuron receives relatively few types of synaptic inputs. Briefly, the auditory nerve bifurcates upon entering the brainstem, sending one process to nucleus magnocellularis (NM) and another to nucleus angularis. In NM, the auditory nerve fibers form large synaptic arrangements, called endbulbs of Held, on the soma of NM neurons [4,13,19,32]. Each NM neuron then projects bilaterally to nucleus laminaris (NL) where branches form synaptic boutons on the dendrites of several NL neurons [3,31,45]. Both the auditory nerve-to-NM and the NM-to-NL projections provide excitatory drive to the postsynaptic neurons which are mediated by amino acid neurotransmitters [26,29,42,47].

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The powerful excitatory input into NM provided by the auditory nerve is presumed to be important for relaying auditory information accurately and rapidly to higher order neurons. However, NM neurons also receive non-auditory nerve synaptic contacts [30]. Previous anatomical studies have shown that these synaptic contacts contain the inhibitory neurotransmitter γ-amino-butyric acid (GABA) [6,8], and receptor binding assays [9] have shown that $GABA_a$ receptors are present in NM. A major portion of the GABAergic input arises from a bilateral projection from the superior olivary nucleus [23]. GABAergic cells projecting into the NM and NL are also scattered between the two nuclei [44]. These GABAergic inputs may act to modify information relayed through NM neurons from the auditory nerve.

GABA is one of the primary inhibitory neurotransmitters in the central nervous system. The GABA receptor systems are generally categorized into two types: $GABA_a$ and $GABA_b$ (for review, see [41]). Activation of $GABA_a$ receptors opens chloride channels which, depending on the relative concentrations of internal and external Cl^-, typically results in a hyperpolarizing response. Activation of $GABA_b$ receptors typically results in a hyperpolarization that is mediated by the opening of K^+ channels. These two receptor subtypes can be readily distinguished pharmacologically: $GABA_a$ receptors are activated by the agonist muscimol and blocked by the antagonist bicuculline, whereas $GABA_b$ receptors are selectively activated by baclofen and can be inhibited by agents such as phaclofen.

This report examines the action of GABA on NM neurons and begins to describe the pharmacological profile of the postsynaptic receptors. Intracellular recordings were made using an in vitro slice preparation of the chick brainstem. The response of auditory neurons to exogenously applied GABA agonists were analyzed. For comparison, neurons in the neighboring medial vestibular nucleus (VeM) were also examined. Our results indicate that an unusual type of GABA receptor is present on neurons in the brainstem auditory system. GABA application to these neurons resulted in a depolarizing, inhibitory response. Although this type of response was also observed to the $GABA_a$ agonist, muscimol, it was not blocked by the $GABA_a$ antagonist, bicuculline. The response was not evoked by the $GABA_b$ agonist, baclofen, and was not blocked by the $GABA_b$ antagonist phaclofen.

2. Materials and methods

2.1. Brain slice preparation

Brain slices were obtained from chicks 5–14 days posthatch. Chicks were hatched from eggs obtained from commercial suppliers and reared in communal brooders at either the University of Washington or the Florida State University. Three strains of chickens were used: White leghorn, Cobbs Avian, and Ross × Ross. Data obtained from each strain were similar.

The brain slice preparation has been described in detail previously [17,20]. Briefly, the brainstem containing NM and NL was removed and coronal slices were made using a vibratome. Slices were 300 μm thick for current clamp experiments and 50–100 μm thick for whole-cell voltage clamp experiments. Slices were submerged in oxygenated artificial cerebrospinal fluid (ACSF) at 34° C. The media flowed through the recording chamber at approximately 3.5 ml/min.

The ACSF contained 130 mM NaCl, 3 mM KCl, 2 mM $CaCl_2$, 2 mM $MgSO_4$ (or $MgCl_2$), 26 mM $NaHCO_3$, 1.25 mM NaH_2PO_4 and 10 mM D-glucose. The pH was 7.3. ASCF containing low (0.5 mM) Ca^{2+} and high (5 mM) Mg^{2+} concentrations were used in some experiments in order to eliminate synaptic transmission. All media components were obtained from Fisher Scientific.

2.2. Agonists and antagonists

The $GABA_a$ antagonist, bicuculline methiodide (50–100 μM), or the $GABA_b$ antagonist, phaclofen (500 μM) were added to the bath in some experiments. GABA, muscimol hydrobromide and baclofen (1–100 mM) were dissolved in ACSF for focal application. The pharmacological agents were obtained from Sigma except for muscimol (Research Biochemicals Int.).

GABA, muscimol, or baclofen were applied by pressure ejection into the media flowing over the slice. The drugs were pressure ejected into the media through a pipette having a 2–5 μm tip using a wide range of time and pressure settings (Picospritzer II, General Valve). In most experiments, a small amount of the inert dye (Phenol red) was added to the drug solution to visualize drug flow. No reliable responses occurred when Phenol red was injected alone.

2.3. Intracellular recordings

Current clamp. The methods for these experiments are diagramed in Fig. 1. Individual neurons in both NM ($n = 90$) and NL ($n = 6$) were impaled with microelectrodes (60–70 MΩ) containing 4 M potassium acetate. For comparison, the response to GABA application was also recorded in neurons located in the medial vestibular nucleus (VeM; $n = 25$). Only cells having resting potentials more negative than −50 mV were investigated. NM, NL, and VeM neurons were identified based on location and electrophysiological properties. In most cases 'healthy' NM neurons were driven synaptically after electrical stimulation of the auditory

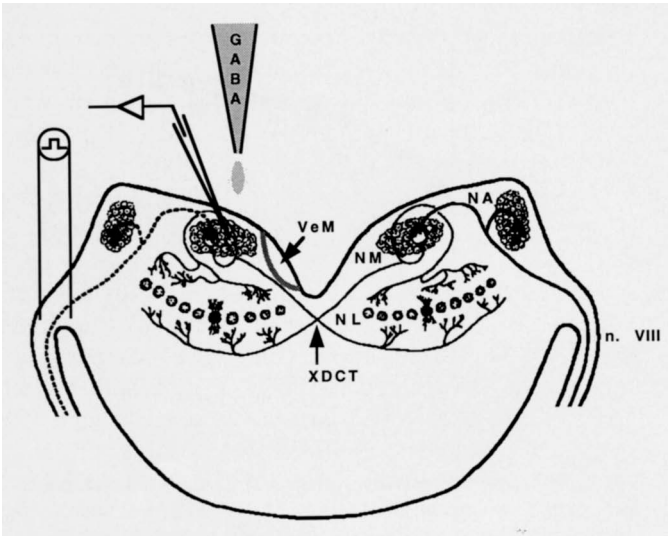

Fig. 1. Schematic of the brainstem auditory system of the chick displaying the experimental protocol. Recordings were made from neurons in the nucleus magnocellularis (NM), nucleus laminaris (NL) or medial vestibular nucleus (VeM). A stimulating electrode was placed on the auditory nerve (n. VIII), as shown, or on NM fibers in the crossed dorsal cochlear tract (XDCT). GABA was ejected from a pipette into the media flowing over the slice.

nerve. As reported previously [13], NM neurons show only one or two levels of EPSP amplitude before reaching threshold for an action potential. For both NM and NL neurons, long depolarizing current pulses produce only one action potential [34]. The action potentials of NL neurons tend to have a longer after-hyperpolarization than NM neurons and a smaller amplitude. EPSPs in NL neurons can be driven by electrical stimulation of NM on either side of the slice [15]. VeM neurons are easily distinguished from the auditory neurons by electrophysiological characteristics as well as by location. The VeM is a visually distinct nucleus located medial to the NM and NL. Long depolarizing current pulses produce a train of action potentials in these neurons and they are not synaptically driven after stimulation of the auditory nerve or NM. VeM neurons often show spontaneous activity.

Input resistance was measured from the voltage response to positive and/or negative current pulses. GABA-mediated inhibition was further examined by evaluating the drug's effect on the generation of action potentials driven either by orthodromic stimulation, antidromic stimulation or intracellular injection of depolarizing current pulses. These action potentials were driven using stimulating electrodes, constructed from a pair of Teflon-coated silver wires, placed either on the auditory nerve (for orthodromic stimulation of NM neurons) or on the axons of NM fibers (for antidromic stimulation of NM neurons or orthodromic stimulation of NL neurons). The constant current (0.1–0.5 mA) stimulation pulses were 20 μs in duration and were presented at 1–10 Hz.

Whole-cell voltage clamp. In some experiments, whole-cell voltage clamp was used to measure the reversal potential. Whole-cell recordings of NM neurons ($n = 7$) were obtained using patch pipettes filled with 10 mM HEPES, 3 mM $MgCl_2$, 140 mM K-gluconate, 2 mM NaCl, 2 mM Na_2-ATP. To maintain pH at 7.2, 0.04 ml of 1 M KOH was added to 5 ml of intracellular solution (see [34]). The ACSF was the same as used in the current clamp experiments. The response to GABA application was measured at a variety of holding potentials. All voltage traces have been corrected for a measured junction potential of -10 mV.

3. Results

3.1. Application of GABA to NM and NL neurons

The consistently observed response to GABA in NM and NL neurons was a depolarization accompanied by a decrease in input resistance. This type of response was observed in every NM and NL neuron. Fig. 2 shows the voltage response of an NM neuron when hyperpolarizing current pulses were injected through the recording electrode and GABA was administered into the bathing medium flowing over the slice. The magnitude and duration of this response depended on the 'dose' of GABA. In this experiment, the dose was manipulated by changing the duration of the pressure pulse ejecting the GABA. Similar dose dependence was also observed by changing the concentration of drug in the pipette, but quantitative comparisons of dilutions proved difficult because of differences in tip size and placement of the drug pipettes. Depending on dose, the depolarization induced by GABA application could be as much as 20 mV. The inset of Fig. 2 shows a similar depolarizing response recorded from a neuron in NL. Fig. 2 shows that change in input resistance is also dose dependent. Current–voltage curves for one cell before, during, and following the GABA depolarization are shown in Fig. 3. A shift in the I–V relationship was observe across a large range of negative current pulses.

The depolarizing effects of GABA never evoked an action potential. Rather, GABA application inhibited action potentials driven by other means. In Fig. 4 (top), the action potential was orthodromically evoked by a current pulse to the auditory nerve. GABA application reduced the likelihood of driven action potentials and only the underlying postsynaptic potential could be observed. Similar results were obtain when action potentials were driven antidromically by stimulating of the NM neuron's axons (Fig. 4, bottom). In this case the spike amplitude was substantially attenuated by GABA application. Action potentials evoked by

suprathreshold current pulses injected into the neuron were also inhibited by GABA (not shown).

Since GABA was applied to the bathing medium it was possible that the observed response was an indirect effect. For example, GABA could act at receptors on an interneuron which, in turn, synapse on the NM or NL neuron. To determine if the effect of GABA on NM and NL neurons was due to a direct action on GABA receptors of the impaled cell, recordings were made while preventing synaptic transmission by maintaining the slice in ACSF containing low Ca^{2+} and high Mg^{2+} concentrations [17] ($n = 6$). This medium had no effect on either the amplitude or duration of the GABA-induced depolarization, but completely eliminated synaptic transmission from the auditory nerve to NM (see Fig. 5). This indicates that the effect

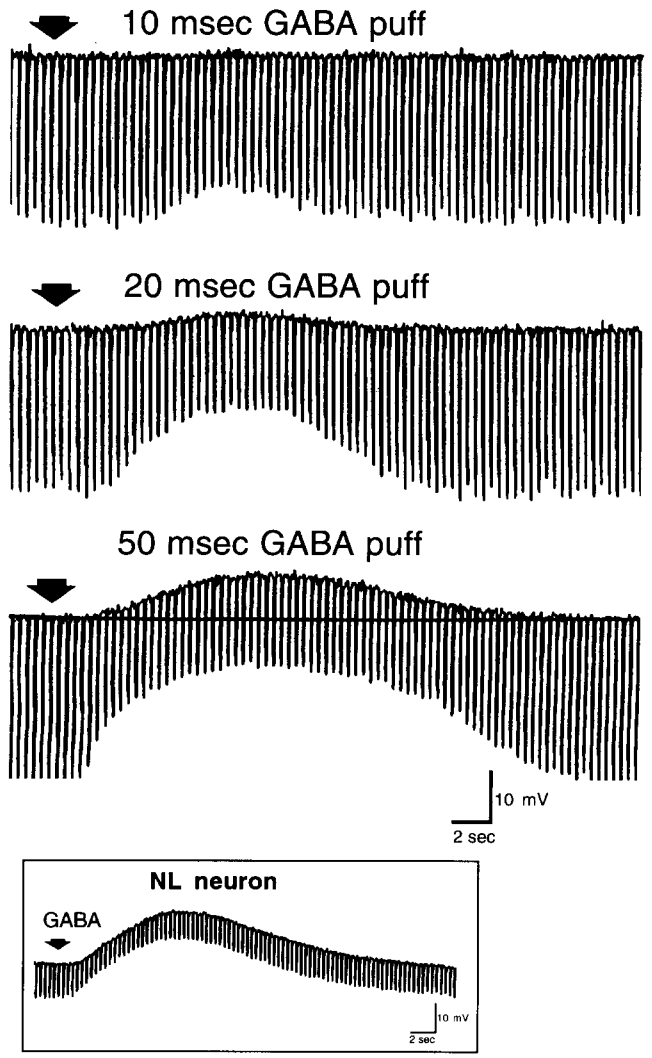

Fig. 2. Dose-dependent depolarizing response to GABA in NM neuron. Arrow indicates the time at which the GABA was ejected into the bath. Dose was varied by changing the duration of the pressure pulse used for ejecting the GABA. The magnitude of the depolarization was greater when longer pulses were used. In every case, auditory neurons showed a depolarizing response to GABA. Downward deflections are voltage responses to hyperpolarizing current pulses (−0.6 nA) injected through the recording pipette. A horizontal reference line is added to the bottom trace at resting potential (−66 mV). Inset: a similar depolarizing response is observed in NL neurons (resting potential = −69 mV; current injection = −0.3 nA).

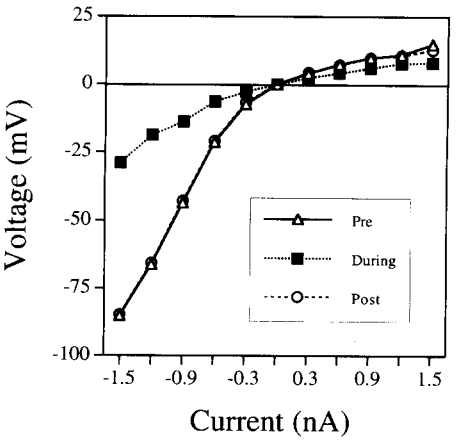

Fig. 3. An example of current–voltage relationships recorded before, during and following the GABA-induced depolarization. Steady-state voltage measurements were taken near the end of 300 ms current pulses. The decrease in input resistance was observed over a wide range of current pulses and the change in input resistance was reversible (resting potential: = −61; membrane potential during depolarization = −54).

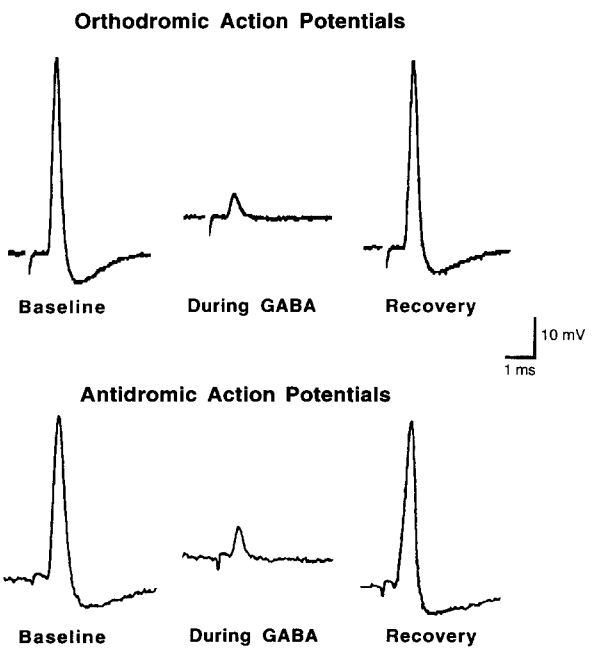

Fig. 4. Reversible inhibition of action potentials by GABA application. Top: action potentials (left) evoked by electrical stimulation of the auditory nerve were eliminated after GABA application (middle). The action potential returned (right) when GABA was washed out (within 1 min after the application). Bottom: similarly, action potentials driven by antidromic stimulation of the NM neuron's fibers were severely attenuated by GABA application. Resting potential = −64 mV.

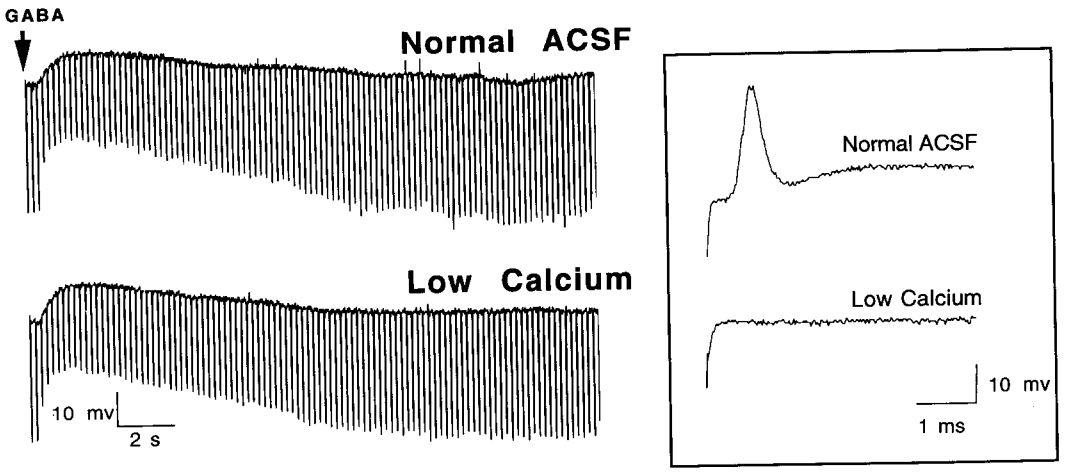

Fig. 5. GABA is acting directly on the NM neuron. The duration and amplitude of the GABA-induced depolarization were unchanged when synaptic transmission was blocked by maintaining the slice in low-Ca^{2+}/high-Mg^{2+} medium. Traces in the box show that the low-Ca^{2+} medium eliminates the postsynaptic potential evoked by stimulating the auditory nerve. This suggests that the GABA-induced depolarization is not mediated by presynaptic mechanisms or via interneurons (resting potential = −61 mV; current injection = −1.5 nA).

of GABA is directly on the neuron under study and not through an interneuron.

Although the depolarizing inhibitory response to GABA was observed in both NM and NL of the brainstem auditory system, it was not observed in neighboring brainstem neurons in VeM. Neurons in the VeM responded to GABA with a hyperpolarization or a biphasic response of depolarization followed by hyperpolarization. In both cases there was an associated decrease in input resistance (see Fig. 7b). Many VeM neurons are spontaneously active. Hence, it was often difficult to determine the precise value of the cells' resting potential. However, GABA application eliminated the spontaneous action potentials and the subsequent shift in the membrane potential was apparent.

3.2. Pharmacology

To distinguish between $GABA_a$ and $GABA_b$ subtypes of receptors, specific agonists and antagonists were applied. The $GABA_a$ agonist, muscimol ($n = 10$), produced a depolarizing, inhibitory response similar to that produced by GABA (Fig. 6). In contrast, the $GABA_b$ agonist, baclofen ($n = 6$) evoked neither a depolarization nor a decrease in input resistance. In both cases 0.1 M concentrations of the agonist were applied through by pressure ejection into the media flowing over the slice. At high doses (i.e. long puff durations), baclofen did inhibit synaptically driven action potentials, but this is likely attributable to a presynaptic effect on the auditory nerve terminals since there was no change in membrane potential or input resistance. In cells tested with two different agonists, baclofen failed to evoke changes in membrane potential, whereas a depolarizing response to either GABA or muscimol was observed ($n = 3$).

Surprisingly, the $GABA_a$ antagonist, bicuculline, did not reliably alter the response to GABA in NM neurons ($n = 7$), even at doses as high as 100 μM. An example is shown in Fig. 7a. In some cells, however, bicuculline caused an increase in the input resistance even in the absence of GABA application. This suggests the possibility of tonically-open bicuculline sensitive channels. In contrast to the lack of effect in NM, bicuculline completely blocked the GABA-induced response in VeM neurons ($n = 3$, see Fig. 7b). The $GABA_b$ receptor antagonist, phaclofen (500 μM), also had no effect on the GABA response in NM ($n = 4$).

3.3. Voltage clamp

Whole-cell voltage clamp experiments were carried out on NM neurons to provide additional confirmation

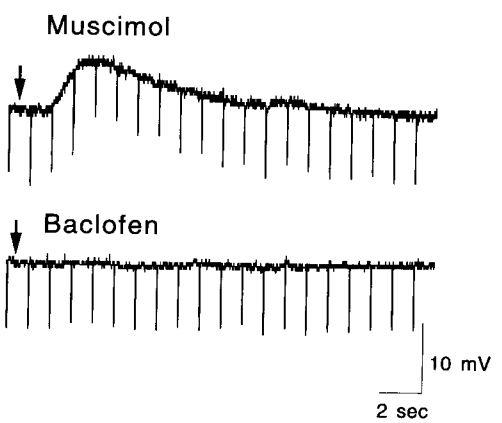

Fig. 6. Depolarizing responses are evoked by $GABA_a$, but not $GABA_b$, agonists. The $GABA_a$ agonist, muscimol, evoked a depolarizing response in NM neurons (top), similar to that observed to GABA. The $GABA_b$ agonist, baclofen (bottom), produced neither a change in membrane potential nor a change in input resistance (resting potential = −65 mV and −66 mV, respectively; current injection = −0.5 nA).

of the GABA-induced response and to determine the reversal potential of this depolarizing response. Under voltage clamp, GABA application resulted in an inward current when the cell was held at resting potential ($n = 7$). These measured responses were similar both with and without 1 μM TTX in the bathing medium. The amplitude of this inward current depended on the level of the holding potential (Fig. 8), and the reversal potential for this current was approximately -45 mV. For comparison, calculated equilibrium potentials for the ions in our preparation are: Na^+ 86, K^+ -103, Cl^- -76. Thus, it appears that the channels opened by GABA in these cells are not selective for a particular ion.

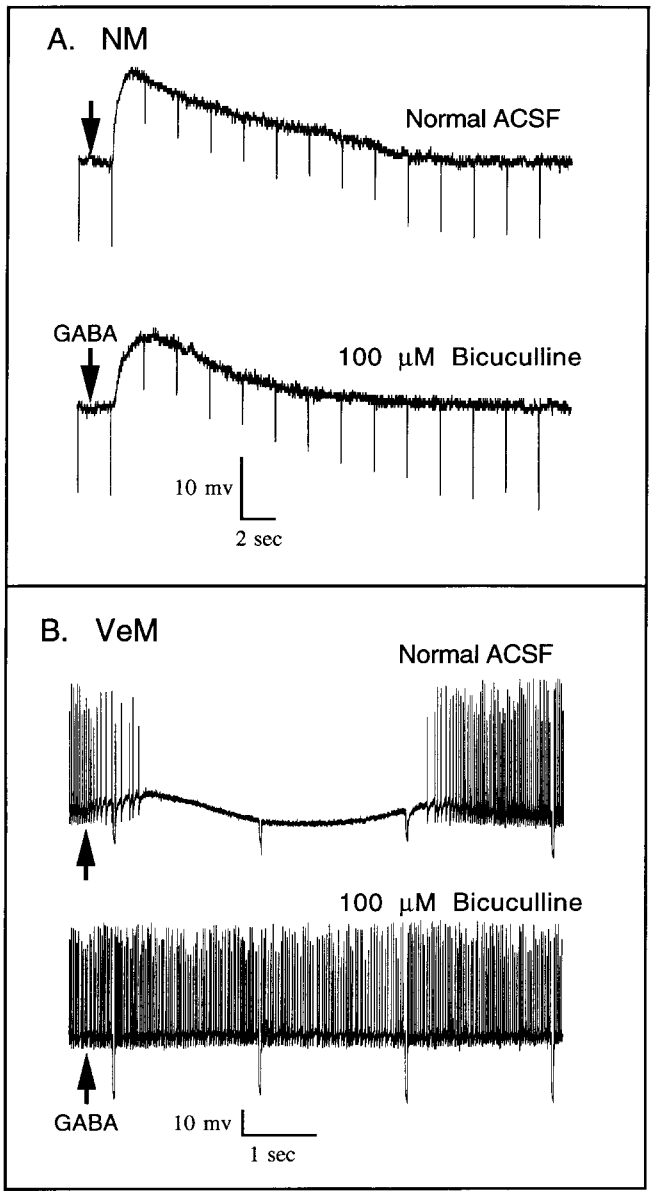

Fig. 7. NM and VeM neurons show different responses to GABA and different sensitivity to bicuculline. A: maintaining the slice in ACSF containing the $GABA_a$ antagonist bicuculline (100 μM) did not prevent the response to GABA in NM neurons (resting potential $= -58$ mV; current injection $= -0.3$ nA). B: the response of neurons in the medial vestibular nucleus (VeM) often showed a biphasic (depolarization–hyperpolarization) response to GABA associated with a decrease in input resistance and a decrease in their spontaneous activity. This response was completely blocked by bicuculline (resting potential ≈ -57 mV; current injection $= -0.5$ nA). Arrows indicate when the GABA was ejected into the media. Downward deflections are voltage responses to hyperpolarizing current pulses injected through the recording pipette. Upward deflections are spontaneous action potentials. Recordings in panels A and B were from cells in the same slice. For clarity, the scales in panels A and B differ.

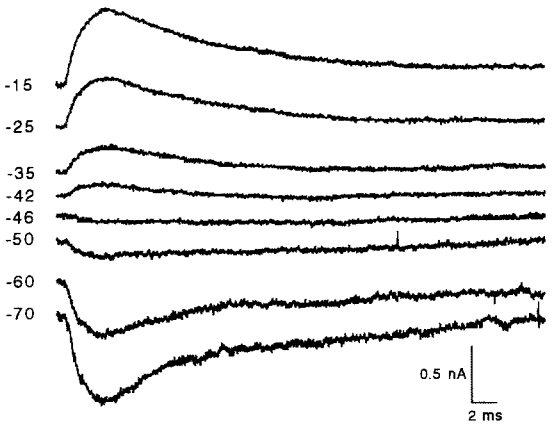

Fig. 8. GABA-evoked currents at various holding potentials for an NM neuron. At resting potential, GABA produced an inward current measured under whole-cell voltage clamp conditions. The reversal potential of the GABA current was approximately -45 mV.

4. Discussion

These experiments demonstrate that brainstem auditory neurons in the chick show an unusual response to application of the inhibitory neurotransmitter GABA. In these neurons, GABA produces a depolarizing, but inhibitory response. Decreasing the Ca^{2+} concentration to levels where synaptic transmission was blocked, produced no reliable change in the GABA response. Thus, this depolarizing response is attributable to a direct action of GABA on the auditory neuron and is not due to of an indirect effect mediated by interneurons.

The depolarizing GABA response does not appear to be mediated by 'standard' $GABA_a$ or $GABA_b$ receptor subtypes. Although a similar response is activated by the $GABA_a$ agonist, muscimol, the $GABA_a$ antagonist, bicuculline, does not block the response. The $GABA_b$ agonist, baclofen, does not evoke a similar response and the $GABA_b$ antagonist, phaclofen, does not block the response. Additionally, the reversal potential of -45 mV suggest that, unlike both the $GABA_a$ and the recently described $GABA_c$ receptor [10,11,33], GABA does not specifically open a Cl^- channel in the brainstem auditory neurons. Thus, neurons in the chick brainstem auditory nuclei appear to

possess a unusual form of GABA receptor. Most likely, this receptor is a variant of the $GABA_a$ receptor.

The unusual properties of the GABA receptor observed in the brainstem auditory neurons are not observed in other GABA receptors of the chick. For example, cultured neurons from the chicks cerebral cortex and spinal cord show bicuculline-sensitive responses with reversal potentials near the equilibrium potential for Cl^- [35]. The unusual receptor apparently is not even a ubiquitous feature of neurons in the chick brainstem. In the same slices, using the same electrodes and the same drug concentrations, neurons in the chick's medial vestibular nucleus show hyperpolarizing (or biphasic) responses that are blocked by bicuculline. These data further confirm that the unusual GABA response observed in brainstem auditory neurons is not simply attributable to methodological variables, such as faulty technique, inadequate drug concentrations, or a defective batch of drug.

Although these experiments were performed on young posthatch chicks, it seems unlikely that the depolarizing GABAergic response is due to immaturity. A large literature suggests that these nuclei are structurally and functionally mature by one to two weeks posthatch (for review, see [36,38]). For example, the distribution of GABAergic terminals in NM changes during embryonic development, but appears to stabilize by early posthatch ages [8]. Nonetheless, this immaturity hypothesis cannot be definitively ruled out, and future developmental analyses of this unusual receptor will be of great interest.

4.1. Other instances of depolarizing GABA responses

Depolarizing responses to GABA are not unique to avian brainstem auditory neurons. For example, depolarizing responses to GABA have previously been observed in the hippocampus [2,18,24,46], visual cortex, [39] neostriatum [28], paratracheal neurons [1], and olfactory cortex [40]. Unlike the depolarizing GABA response observed in the brainstem auditory neurons of the chick, these depolarizing responses were blocked by the $GABA_a$ receptor antagonist, bicuculline.

The ionic fluxes responsible for these depolarizing responses are not fully understood. In many instances, it has been suggested that Cl^- is responsible for carrying the depolarizing GABA current [1,2,27,46]. One hypothesis is that a concentration of ionic pumps, for instance in the dendrite of a CA1 pyramidal cell, results in a local reversal of the chloride gradient. Thus when GABA activates the receptor on the dendrite, chloride ions will flow out of the dendrite resulting in a depolarization. This hypothesis is supported by the results of ion substitution experiments and whole-cell voltage clamp recordings.

If chloride is solely responsible for the depolarizing response to GABA in brainstem auditory neurons, then the mechanism must be somewhat different from that proposed for the hippocampus. At the ages examined, neurons in NM have essentially no dendrite; the GABAergic terminals contact the soma of the cell [8]. Thus the depolarizing GABA response is not attributable to a local reversal of chloride gradients in the cell's dendrites. Our whole-cell voltage clamp recordings from NM neurons indicate that the equilibrium potential of the GABA-induced current is approximately −45 mV, whereas calculations of equilibrium potentials for the various ions in our medium were: Cl^-, −76; Na^+, 86; and K^+, −103. If ionic pumps were able to reverse the Cl^- gradient, they would have to increase the internal chloride concentration of the cell by approximately 3 fold. It seems more likely that the channel responsible for the GABA response is relatively non-selective.

An alternative hypothesis is that the current is carried by HCO_3^- ions. In crayfish muscle cells, GABA activated channels do pass HCO_3^- [22,43]. Since the present experiments used a bicarbonate buffer gassed with 95% O_2/5% CO_2, it is possible that an efflux of HCO_3^- ions could result in the observed GABA-induced depolarization. Using the formulas of Kaila et al. [21] the equilibrium potential for HCO_3^- in our preparation would be approximately −6 mV. However, in other systems, the GABA channel passes both Cl^- and HCO_3^- [5]. If this holds true, then a reversal potential of −45 mV would be possible if the GABAergic channels in the brainstem auditory neurons were just slightly more permeable to Cl^- than to HCO_3^-. Clearly, a number of ion-substitution and pharmacological studies will be required to pinpoint which ions are carrying this GABA-induced current.

Another alternative hypothesis is that this unusual GABA response is mediated by the recently described $GABA_c$ receptor [10,11,33], which is also relatively insensitive to bicuculline. Although this cannot be ruled out at present, two lines of evidence argue against this hypothesis. First, $GABA_c$ receptors appear to open a channel that is selective to Cl^-, whereas this does not appear to be the case for the GABA response in NM neurons (see above). Second, receptor-binding assays using [^{3}H]muscimol [9] and in situ hybridization studies of receptor subunits [14] suggest that the receptors in NM and NL are a variant of the $GABA_a$ receptor.

4.2. Significance of depolarization-induced inhibition

Endbulbs of Held provide a powerful form of excitation from the auditory nerve to NM neurons. Stimulation of the auditory nerve afferents can produce unitary EPSPs that can exceed 20 mV [13]. If an inhibitory pathway were to be effective, it would also have to provide a equally powerful mode of inhibition. A rela-

tively small hyperpolarization by opening of chloride channels might be insufficient to block this powerful excitatory drive. A mechanism suggested by the present results may serve to be a more efficient means of inhibition. First, the depolarizing response produced by GABA could inactivate the voltage-dependent Na^+ channels. The observed reduction of the antidromically driven action potential supports this hypothesis. In addition, Reyes et al. [34] have shown that sustained depolarization can inactivate the action potential mechanism in NM neurons. Second, because the reversal potential of the GABA response is relatively near resting potential, it can shunt part of the excitatory input. Finally, the depolarization produced by GABA is sufficient to activate the fast activating and slowly inactivating potassium current that is present in NM and mammalian cochlear nucleus neurons [25,34] This current is activated at subthreshold levels of depolarization and would result in an additional decrease in input resistance, thus making subsequent EPSPs less effective.

The role of this GABAergic inhibition in the processing of acoustic information is still unknown. Anatomical studies have shown that GABAergic terminals and receptors are abundant in both avian and mammalian brainstem auditory systems. Immunolabeling studies have shown a greater density of GABAergic terminals in low frequency regions of NM than in high frequency regions [8]. The current studies showed a depolarizing inhibitory response to GABA in every NM neuron studied, but the methods would not allow any comparison of GABA sensitivity in different regions of the nucleus. Thus the functional role of this anatomical gradient is still unclear.

A portion of the GABAergic input to the auditory nuclei originates from neurons that are scattered between and around NM and NL. These neurons are immunolabeled by an antibody that recognizes GABA and these cells appear to send processes into these auditory nuclei [44]. Given the close proximity to NM and NL, it is possible that some of these GABAergic cells were impaled in the present study. No distinct population was noted, however, on the basis of their firing pattern or in their response to GABA application. Cells located in the superior olive provide a major source of the GABAergic input [23]. It will be of great interest to undertake direct tests of GABA's role in information processing within this circuit.

One indirect test of the role of GABA in auditory system function has been made in the barn owl. Fujita and Konishi [12] ionophoresed bicuculline into the area of NL while recording from the inferior colliculus. They observed that bicuculline administration broadened the tuning of IC units to interaural time differences. Thus it is possible that GABA plays a role in coding the location of an acoustic stimulus. These data are somewhat at odds with those from the current set experiments, however, in that bicuculline failed to block the GABA-induced response in the chick. There are a number of potential explanations for this apparent discrepancy. First, although GABA produced depolarizing responses in both NM and NL, our bicuculline tests were only performed on NM neurons. It is possible that the response in NL is bicuculline-sensitive. Second, although the GABA-induced response was not blocked by bicuculline, an increase in input resistance was sometimes noted when a large amount of bicuculline was added to the medium. Clearly, this could alter the response properties of NL neurons. Third, it is possible that the ionophoretic injections into NL in vivo affected coding by blocking GABA receptors on interneurons rather than NL neurons per se. Finally, the apparent discrepancy may simply be attributable to species differences or the ages of the animals used in the two studies.

In addition to its likely role in synaptic inhibition, GABA could possibly have a trophic function in this system. Cherubini et al. [7] have suggested that GABA-induced depolarizations early in life are the primary source of excitation and could serve a trophic role by setting into motion a cascade of events associated with a rise in intracellular Ca^{2+}. Additionally, a trophic role could be mediated by intracellular changes in pH if GABA caused an efflux in HCO_3^- [5,21,22,43]. Along these lines, numerous studies have shown that the structural and metabolic integrity of NM neurons in the chick is dependent on afferent activity [37]. For example, removal of auditory nerve activity results in the eventual death of a portion of neurons and the remainder shrink in size. At present, the precise neurochemical signals involved in this form of transneuronal regulation are not known. Some evidence suggests that activation of excitatory amino acid receptors is necessary for these effects [16], but GABAergic receptors could still play a primary or cooperative role.

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