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MODULATION OF CALCIUM BY INHIBITORY SYSTEMS IN THE DEVELOPING AUDITORY MIDBRAIN

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Abstract—Inhibitory synaptic transmission is of fundamental importance during the maturation of central auditory circuits, and their subsequent ability to process acoustic information. The present study investigated the manner in which inhibitory transmission regulates intracellular free calcium levels in the gerbil inferior colliculus using a brain slice preparation. Inhibitory and excitatory postsynaptic potentials were evoked by electrical stimulation of the ascending afferents at the level of the dorsal nucleus of the lateral lemniscus. Pharmacologically isolated inhibitory synaptic potentials were able to attenuate a calcium rise in collicular neurons that was generated by depolarizing current injection. In addition, $GABA_A$ and glycine receptor antagonists typically led to an increase of calcium in collicular neurons during electrical stimulation of the ascending afferent pathway at the level of the dorsal nucleus of the lateral lemniscus. Bath application of GABA or muscimol, a $GABA_A$ receptor agonist, evoked a brief hyperpolarization followed by a long-lasting depolarization in inferior colliculus neurons. This treatment also induced a transient calcium increase that correlated with the membrane depolarization phase. Baclofen, a $GABA_B$ receptor agonist, had no effect on either membrane potential or calcium levels. Ratiometric measures indicated that the muscimol-evoked rise in calcium was ≈ 150 nM above basal levels. The muscimol-evoked responses were completely antagonized by bicuculline and attenuated by picrotoxin.

Together, these results suggest that inhibitory synaptic transmission participates in the regulation of postsynaptic calcium during the developmental period. Inhibitory transmission may attenuate a calcium influx that is evoked by excitatory synapses, but it can also produce a modest influx of calcium when activated alone. These mechanisms may help to explain the influence of inhibitory transmission on the development of postsynaptic properties. © 1998 IBRO. Published by Elsevier Science Ltd.

Key words: synaptic inhibition, calcium, inferior colliculus, muscimol, GABA, glycine, plasticity.

The inferior colliculus (IC) is an obligatory processing station for most acoustic information, and it receives inhibitory and excitatory afferents from the majority of brainstem auditory nuclei.[26,46] Substantial evidence has demonstrated that synaptic inhibition is essential for auditory processing in the IC. There is a wide range of functional consequences when $GABA_A$ or glycine receptor antagonists are iontophoretically applied while recording sound-evoked responses from the IC, These include enhanced sound-evoked discharge rates, modification of rate–intensity functions, changes in response latency, perturbations of binaural processing, broadening of frequency tuning, and elimination of duration tuning.[10,15,16,48,49,71,76] Therefore, the cellular events that accompany inhibitory synaptic transmission are of considerable interest.

Among the several ascending inhibitory projections to the IC, the projection from the dorsal nucleus of the lateral lemniscus (DNLL) is considered to be important for binaural processing.[17,33] Evidence from both anatomical and functional studies indicate that DNLL is a major source of extrinsic GABAergic afferents to the IC. Most DNLL neurons are labelled with antibodies directed against glutamic acid decarboxylase and GABA,[1,42,51,68] and their axons project into the ipsi- and contralateral IC.[37,62 64,78] At a functional level, pharmacological activation or inactivation of DNLL modulates binaural processing in a manner consistent with an inhibitory projection.[17,33]

GABA is commonly recognized as the most widely distributed inhibitory neurotransmitter in the CNS of vertebrates. However, it has also been shown that GABAergic and glycinergic transmission can depolarize neurons, and may lead to an increase of intracellular free calcium $[Ca^{2+}]_i$.[3,11,12,19,25,28,40,60] These results raise the possibility that GABAergic systems regulate the maturation of postsynaptic neurons by modulating their $[Ca^{2+}]_i$ levels.

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Abbreviations: ACSF, artificial cerebrospinal fluid; AP5, D(−)-2-amino-5-phosphonopentanoic acid; $[Ca^{2+}]_i$, intracellular calcium; CNQX, 6-cyano-5-nitroquinoxaline-2,3-dione; DNLL, dorsal nucleus of the lateral lemniscus; EGTA, ethyleneglycolbis(aminoethylether)tetra-acetate; EPSP, excitatory postsynaptic potential; F, fluorescence; F_o, initial fluorescence; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid; IC, inferior colliculus; IPSP, inhibitory postsynaptic potential; LSO, lateral superior olive.

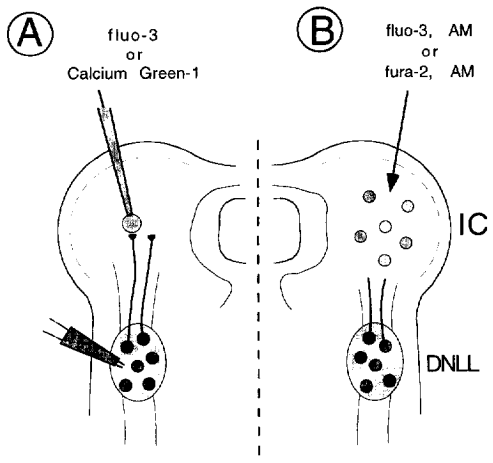

Fig. 1. Schematic of the DNLL-IC brain slice preparation. Two techniques were employed. In the first set of experiments, brain slices were incubated in membrane permeant calcium indicator dyes (right). In the second set of experiments, whole-cell recordings were obtained in the central nucleus of the IC with electrodes that contained a calcium indicator dye (left). In some experiments, electrical stimuli were applied to the dorsal aspect of DNLL.

Our previous experimental findings suggest that synaptic inhibition plays an important role in regulating functional and structural maturation in the lateral superior olive (LSO).[4,31,55 57,59] Since GABAergic inhibition is of such general importance to auditory processing in the IC, and may also serve as a developmental signal, we examined how inhibitory neurotransmission modulates intracellular Ca^{2+} levels in a brain slice preparation using whole-cell recordings in combination with calcium imaging.

EXPERIMENTAL PROCEDURES

Brain slice preparation

Brain slices were prepared following previously described procedures.[53,54] Briefly, gerbils (*Meriones unguiculatus*; Tumblebrook Farms) aged nine to 15 days postnatal were deeply anaesthetized (300 mg/kg chloral hydrate), decapitated, and 300 μm Vibratome sections through the auditory brainstem were obtained. All efforts were made to minimize animal suffering and to reduce the number of animals used, and the protocols were approved by the NYU Animal Care and Use Committee. The slices were preincubated in oxygenated (95% O_2/5% CO_2) artificial cerebrospinal fluid (ACSF: 123 mM NaCl, 4 mM KCl, 1.2 mM KH_2PO_4, 1.3 mM $MgSO_4$, 28 mM $NaHCO_3$, 15 mM glucose, 2.4 mM CaCl, 0.4 mM ascorbic acid) at room temperature for 12 h before recording.

Electrophysiological recording

The brain slice containing IC and DNLL (Fig. 1) were transferred to a custom-designed recording chamber. Oxygenated ACSF was superfused through the recording chamber at 6–8 ml/min, and the preparation was kept at room temperature. The whole-cell current-clamp configuration was used to record from IC neurons.[30,31] Non-fire-polished electrodes of 5–8 MΩ were fabricated from borosilicate glass (1.5 mm) on a multi-cycle puller (Sutter Instruments), and backfilled with an internal pipet solution containing (in mM): 130 K-gluconate, 0.6 EGTA, 10 HEPES, 2 MgCl, 5 KCl, 2 ATP, 0.3 GTP, pH=7.2. As illustrated in Fig. 1A, the bipolar stimulating electrodes were place on the ipsilateral DNLL or the DNLL afferents to IC. Data was acquired with an AxoClamp 2A amplifier interfaced to a PC 486 using Axotape2 software, and analysed with pClamp6 software (Axon Instruments).

In some cases the following chemicals were dissolved in oxygenated ACSF and superfused through the brain slice chamber: GABA (Sigma Chemical; 200 μM), glycine (200 μM; Sigma Chemical), glutamate (100 μM; Sigma Chemical), muscimol (50–100 μM; RBI), 6-cyano-5-nitroquinoxaline-2,3-dione (CNQX; Tocris; 10 μM), D(−)-2-amino-5-phosphonopentanoic acid (AP5; Tocris; 25 μM), picrotoxin (Sigma Chemical; 50 μM), bicuculline (Sigma Chemical; 100 μM), and strychnine (Sigma Chemical; 5 μM). These drug concentrations were chosen during an initial set of pilot experiments, and they represent the lowest concentrations that reliably elicited a change in fluorescence using Fluo-3 AM.

Calcium indicator dye imaging

To monitor $[Ca^{2+}]_i$ during electrophysiological recordings, IC neurons were passively loaded with either Fluo-3 (100 μM) or Calcium Green-1 (62.5 μM) via the recording pipette for 10 min (Fig. 1A). To monitor $[Ca^{2+}]_i$ in several neurons simultaneously, slices were incubated in 5 μM Fluo-3 AM or Fura-2 AM for 45 min, and then washed in ACSF at 31°C for 30 min (Fig. 1B). To make the working incubation solution, 50 μg of indicator was dissolved in 20% pluronic acid/dimethylsulphoxide, and then combined with ACSF.

Cells were visualized with either a 10× (non-ratiometric measures) or a 40× water immersion (ratiometric measures) objective (Nikon Optiphot-2). Neutral density filters were used to minimize dye bleaching and phototoxicity. For non-ratiometric imaging with Fluo-3 or Calcium Green-1, a 475–500 nm exciting wavelength filter was used. For ratiometric imaging with Fura-2, 350 and 380 nm excitation wavelength filters were used. Pairs of images were collected at 0.5 Hz, and each image was averaged from eight video frames. Fluorescent signal was obtained with a GenSysII intensifier and CCD72 camera (Dage-MTI). Acquisition software (Ratiotool, Inovision) was used to store and analyse the images.

Data collected for non-ratiometric imaging (Calcium Green-1 or Fluo-3) were collected at 0.5 Hz, and analysed as the percentage change in fluorescence ($F-F_o/F_o$; F, fluorescence; F_o, initial fluorescence). Ratiometric data (Fura-2) was converted to calcium concentration (nM). To determine calcium concentration, calibrations were performed with a standard Ca^{2+} buffer kit and Fura-2 pentapotassium salt (Molecular Probes) and ratios were subsequently converted using the formula.[21]

$$[Ca^{2+}]_i = K_d((R - R_{min})/(R_{max} - R)) \cdot (S_{f2}/S_{b2})$$

A K_d value of 135 nM was used since experiments were conducted at room temperature. All data were analysed except ratios that were on the non-linear portion of the calibration curve (i.e. extremely large responses to glutamate that saturated the detector).

RESULTS

The effect of inhibitory synaptic activity on Ca^{2+} signals

When the DNLL was stimulated electrically, both excitatory (EPSPs) and inhibitory post-synaptic potentials (IPSPs) were observed, and they

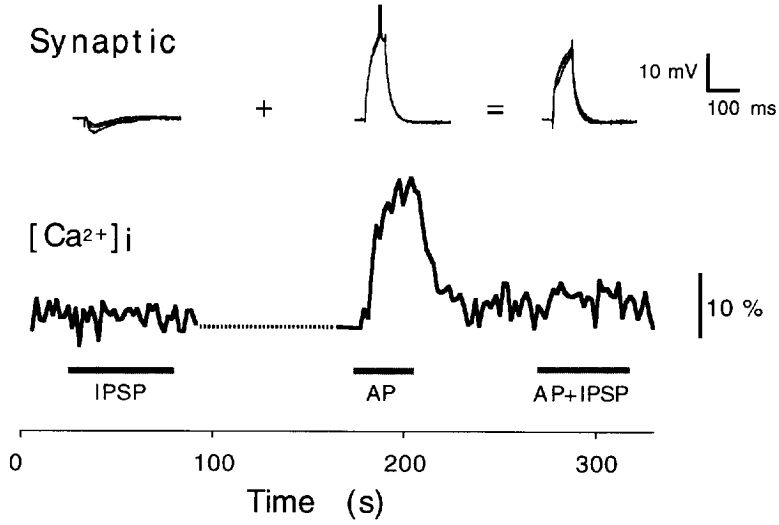

Fig. 2. IPSPs block current-evoked action potentials and attenuate the attendant rise in $[Ca^{2+}]_i$. (Top) Traces show examples of DNLL-evoked IPSPs (in the presence of AP-5 and CNQX), intracellular current-evoked action potentials, and the conjunctive presentation of IPSPs with action potentials (from left to right). The action potentials are clipped off in the middle set of traces. (Bottom) The continuous trace shows the percent change in calcium indicator (Calcium Green-1) fluorescence ($F-F_o/F_o$) during each of the manipulations. The evoked IPSPs at 2 Hz had no effect on $[Ca^{2+}]_i$ levels, while action potentials elevated $[Ca^{2+}]_i$. This current-evoked $[Ca^{2+}]_i$ transient was completely blocked by IPSPs. AP, action potential.

commonly producing a biphasic response. This undoubtedly reflects the fact that lemniscal fibres from superior olivary and cochlear nuclei course directly through the DNLL. To test whether the inhibitory synaptic component normally acts to constrain the influx of Ca^{2+}, pure IPSPs were evoked by stimulating DNLL in the presence of 10 μM CNQX and 25 μM AP-5. As shown in Fig. 2, a low rate of afferent-evoked IPSPs did not produce a significant change in $[Ca^{2+}]_i$. However, depolarizing, current pulses elicited action potentials, and led to a significant rise in $[Ca^{2+}]_i$. When IPSPs were delivered simultaneously with these same depolarizing current pulses, action potentials were blocked and the $[Ca^{2+}]_i$ signal was attenuated. The current-evoked rise in Fluo-3 fluorescence ($F-F_o/F_o$) was $14\pm2\%$ (Mean ± S.E.M.; $n=12$), but the rise was only $4\pm2\%$ when IPSPs and current injection were evoked simultaneously. In every case, the ability of IPSPs to attenuate a rise in $[Ca^{2+}]_i$ was associated with a reduced number of current-evoked action potentials (Fig. 2).

We next examined whether IPSPs also limit the rise in postsynaptic $[Ca^{2+}]_i$ that is produced by excitatory synaptic activity. A complex excitatory/inhibitory synaptic response was first evoked with DNLL stimulation and $[Ca^{2+}]_i$ was monitored (Fig. 3A). Synaptic inhibition was then blocked with both $GABA_A$ and glycine receptor antagonists, picrotoxin (50 μM) and strychnine (5 μM). In the presence of picrotoxin and strychnine, synaptically-evoked depolarizations and postsynaptic $[Ca^{2+}]_i$ levels were each enhanced (Fig. 3A). As shown in Fig. 3B, the peak synaptically-evoked rise in Fluo-3 fluorescence was initially $5\pm3\%$, but increase to $10\pm4\%$ in the presence of antagonists (t-test, $P<0.005$; $n=9$).

Calcium responses evoked by inhibitory and excitatory agonists

To investigate the direct effect of neurotransmitters or agonists on the cytosolic-free calcium, IC slices were incubated in either Fura-2 AM or Fluo-3 AM. The $[Ca^{2+}]_i$ responses of IC neurons to excitatory or inhibitory neurotransmitters were assessed during exposure to different agents in the recording chamber. Muscimol application (50 μM) increased the $[Ca^{2+}]_i$ from a resting level of 155 ± 33 nM to 319 ± 65 nM. By comparison, glutamate raised $[Ca^{2+}]_i$ to 753 ± 162 nM ($n=19$, five slices). Figure 4 shows the response in four different neurons from the same slice. In the top two traces the muscimol-evoked rise in $[Ca^{2+}]_i$ was similar or higher than the responses evoked by glutamate. The bottom two traces show neurons that displayed a smaller response to muscimol application, suggesting that the response to muscimol was heterogeneous amongst the IC neurons sampled.

The $[Ca^{2+}]_i$ response was also examined non-ratiometrically with Fluo-3. As shown in Fig. 5, the responses suggest some degree of heterogeneity within the IC, although most neurons did respond to 100 μM glutamate and 50 μM muscimol. Of 33 cells examined, 23 responded to glutamate (100 μM), 28 to muscimol (50 μM), 14 to GABA (200 μM), and five to glycine (200 μM). The magnitude of the

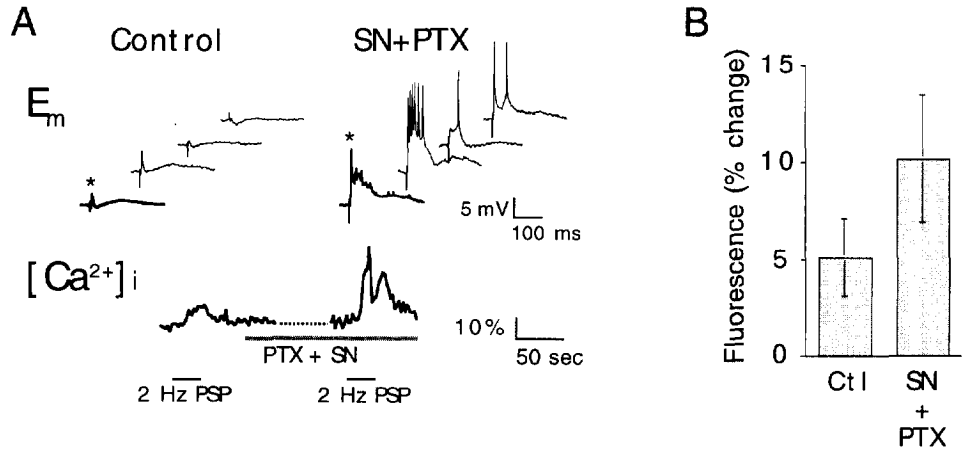

Fig. 3. Blockade of synaptic inhibition increases synaptically-evoked action potentials and the attendant rise in $[Ca^{2+}]_i$. (A) The synaptic and calcium signal from a single IC neuron. (Top) DNLL-evoked synaptic responses are shown. The thick traces (*) show an average from 20 traces, and the three offset traces above them are individual examples. Following the addition of strychnine and picrotoxin, the DNLL-evoked responses produced one to several action potentials. There were initially eight APs from 20 stimulus pulses to the afferent pathway, and after picrotoxin and strychnine treatment this increased to 62 APs from 20 stimuli. (Bottom) The traces show the percent change in calcium indicator (Calcium Green-1) fluorescence during synaptic stimulation ($F - F_o/F_o$). Synaptically-evoked changes in $[Ca^{2+}]_i$ levels were clearly elevated following the addition of SN and PTX. The dashed line indicates a 150 s interval. (B) A bar graph summarizes the effect of blocking inhibition on synaptically evoked rise in $[Ca^{2+}]_i$ from the nine neurons tested (Mean ± S.E.M.). SN, strychnine; PTX, picrotoxin; Ctl, control.

fluorescence change ($F - F_o/F_o$) in response to each compound is summarized in Fig. 5B. The relative efficacy of the agonists in producing a rise in $[Ca^{2+}]_i$ was glutamate>muscimol>GABA>glycine. No cells were found to respond to baclofen (100 μM), suggesting that the Ca^{2+} response to GABA was mediated by a $GABA_A$ receptor mechanism exclusively.

Responses of inferior colliculus neurons to muscimol

The results from membrane permeable calcium indicator dyes (Figs 4, 5) suggested that the GABAergic system could evoke significant increases in free calcium. Therefore, the electrical responses and $[Ca^{2+}]_i$ of IC neurons were monitored concurrently by using whole-cell recordings with Calcium Green in the electrode (see Experimental Procedures). As illustrated in Fig. 6A, muscimol typically produced a brief hyperpolarizing response followed by a longer-lasting depolarization (8.4 ± 0.7 mV, n=11). During the depolarization, the Calcium Green fluorescence signal increased by 15 ± 3% (n=11). The rise in $[Ca^{2+}]_i$ signals generally correlated with the depolarization phase of the muscimol response. However, the temporal resolution of drug perfusion and our calcium imaging system only permitted a crude estimate of timing with respect to the electrophysiology. A decrease of membrane input resistance was also observed during the depolarization, changing from a pretreatment mean of 293 ± 17 MΩ to 170 ± 12 MΩ during exposure to muscimol (n=8).

To test whether the muscimol-induced rise in $[Ca^{2+}]_i$ was simply due to membrane depolarization, the muscimol-evoked response was mimicked with a depolarizing current injection through the recording pipette (Fig. 6B). While Ca^{2+} fluorescence levels were elevated by the depolarization (4 ± 1%, n=6), the effect was less than that produced by the muscimol-evoked depolarization. During current injection, the input resistance decreased from a pretreatment mean of 256 ± 28 MΩ to 229 ± 38 MΩ during the depolarization (n=4).

Pharmacology of the inferior colliculus responses to muscimol

To further assess whether the muscimol-evoked response was mediated by $GABA_A$ receptors, slices were pre-exposed to either 50 μM picrotoxin or 100 μM bicuculline (Fig. 7). These antagonist concentrations were sufficient to suppress GABAergic IPSPs (not shown). In the presence of picrotoxin, muscimol continued to elicit a membrane depolarization (5 ± 1 mV), and an peak increase in Ca^{2+} fluorescence (5 ± 2%; n=8), In contrast, biculline completely blocked both the membrane depolarization and the rise in Ca^{2+} fluorescence (n=7).

DISCUSSION

The present study shows that DNLL-evoked IPSPs can suppress or attenuate the postsynaptic elevation of $[Ca^{2+}]_i$ that is produced by either EPSPs or depolarizing current injection in the developing IC. These results are consistent with studies showing that the projection from the DNLL to the IC is primarily

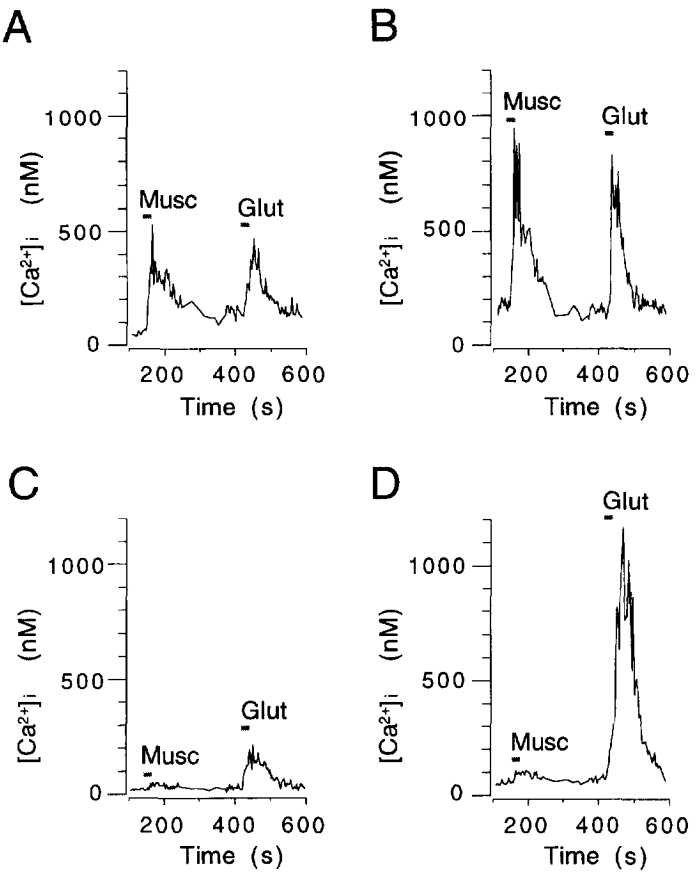

Fig. 4. Comparison of muscimol- and glutamate-evoked $[Ca^{2+}]_i$ increase. (A–D) The response of four cells from the same brain slice are shown (50 μM muscimol, 100 μM glutamate for 45 s indicated by black bars). The data reveal a heterogeneity of response from IC neurons. Some neurons displayed relatively large muscimol-evoked responses in comparison to that elicited by glutamate (A and B). However, other neurons displayed small, but measurable calcium transients in response to muscimol (C and D). There did not appear to be any correlation between resting calcium levels and the magnitude of the response. The calcium concentration was obtained ratiometrically as described in Experimental Procedures. Musc, muscimol; Glut, glutamate.

GABAergic.[1,64] In contrast, it was found that exposure of IC neurons to either GABA or muscimol produced a reversible increase in postsynaptic $[Ca^{2+}]_i$ levels. The implications of these findings for neural development and stimulus processing are discussed below.

Inhibitory suppression of evoked rise in $[Ca^{2+}]_i$

Inhibitory synaptic activity was able to attenuate the $[Ca^{2+}]_i$ rise generated by either depolarizing current or excitatory synaptic activity. Thus, intracellular Ca^{2+} levels are likely to be modulated by the interaction between excitatory and inhibitory synaptic events. This effect may be mediated by shunting the excitatory current and keeping the neuron below the activation threshold of voltage-gated Ca^{2+} channels. For example, inhibitory potentials can attenuate climbing fibre-evoked $[Ca^{2+}]_i$ influx locally in Purkinje cell dendrites.[8] This inhibition is probably mediated by GABA, since it has been previously shown that GABA prevents the generation of climbing fibre-evoked Ca^{2+} action potentials in Purkinje cell dendrites.[24] While results from the present study are consistent with these observations in cerebellum, it is important to note that $[Ca^{2+}]_i$ changes were only monitored at the soma of IC neurons, and no effort was made to pharmacologically isolate GABAergic or glycinergic IPSPs in this specific set of experiments.

GABAergic depolarizations and rise in $[Ca^{2+}]_i$

A growing literature suggests that GABA may acts as an excitatory transmitter by depolarizing cells and elevating intracellular calcium levels in embryonic and early postnatal neurons, both dissociated culture and brain slice preparations.[11,12,29,38,50,61,72,75,77] Such studies have demonstrated that the pharmacology of the depolarizing GABA responses are associated with the GABA receptor. Moreover,

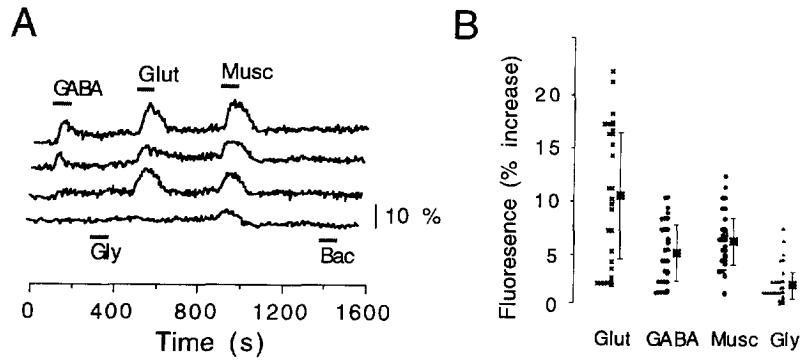

Fig. 5. Effect of different agents on evoking an increase of intracellular Ca^{2+} levels. (A) Increases of $[Ca^{2+}]_i$ are shown as a change in fluorescence of the calcium indicator dye ($F - F_o/F_o$). Most neurons responded to muscimol (100 μM) and glutamate (100 μM), but few neurons responded to glycine (300 μM), and no neurons responded to baclofen (100 μM). The exposure period is indicated by the bars. (B) A summary of responses from 31 IC neurons is shown. Each data point is from an individual cell, and the large asterisk indicates the mean for all neurons (lines represent S.E.M.). A slow negative slope, presumably reflecting dye loss or photobleaching has been subtracted from each trace.

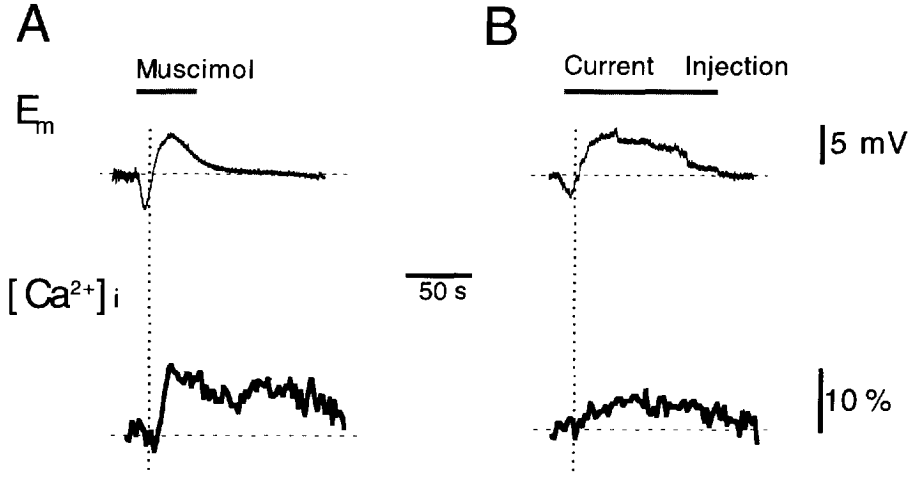

Fig. 6. Comparison of muscimol- and current-evoked changes in membrane potential and $[Ca^{2+}]_i$. (A) Muscimol exposure (50 μM) produced a transient hyperpolarization, followed by a longer-lasting depolarization. The depolarization was associated with an increase in $[Ca^{2+}]_i$. (B) Current was injected into the same IC neuron to mimic the effect of muscimol (top), but the $[Ca^{2+}]_i$ rise was relatively smaller compared to muscimol. The time-courses for muscimol application and current injection is indicated by the bars. The percent change in calcium is given as $F - F_o/F_o$.

glycinergic IPSPs are depolarizing in the rat LSO during the first postnatal week.[28]

While the cellular basis of membrane depolarization is not fully understood, it may be driven by an outward flow of Cl^- through GABA receptor-coupled channels,[45,47,50] or an activity-dependent shift of the $GABA_A$ reversal potential towards the bicarbonate equilibrium potential.[7,27,67] In the mouse barrel cortex, inhibitory postsynaptic current reversal potentials becomes more negative during development as recorded with a whole-cell configuration that presumably establishes the internal chloride concentration.[2] Therefore, it has been suggested that either a chloride uptake mechanism is active or the HCO_3^- permeability of the synaptic channel is relatively large.[41,67] Other candidate mechanisms include the relative efficacy of inwardly rectifying chloride channels,[66] or a sodium-dependent chloride transport mechanisms.[52] The IPSPs recorded in gerbil IC neurons were hyperpolarizing, consistent with a chloride equilibrium potential that was negative to the resting potential (E_{Cl} was -68 mV, and E_{rest} varied between -47 to -60 mV). However, the extent to which the bicarbonate equilibrium potential contributes to the muscimol-evoked depolarization remains to be determined.

Depolarizations elicited by inhibitory activity could activate voltage-gated Ca^{2+} channels, although Ca^{2+} release from internal stores can also contribute to the response.[44] In the present study, muscimol evoked a rise of $[Ca^{2+}]_i$ that occurred during the recorded membrane depolarization, implicating a $GABA_A$ receptor-coupled mechanism. The muscimol-evoked rise in $[Ca^{2+}]_i$ was partially

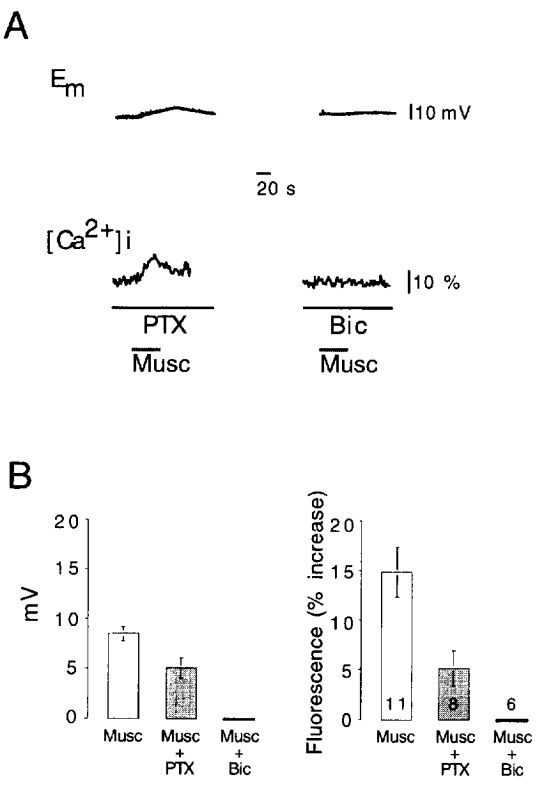

Fig. 7. Relative affect of picrotoxin and bicuculline on the muscimol-evoked response in IC neurons. (A) The muscimol-evoked change of membrane potential (top) and $[Ca^{2+}]_i$ (bottom) were totally suppressed by 100 μM bicuculline (right). While picrotoxin blocked membrane hyperpolarization, it only attenuated membrane depolarization and the $[Ca^{2+}]_i$ rise. (B) The bar graph presents the percent change in membrane potential (mV; grey bars) and $[Ca^{2+}]_i$ ($F-F_o/F_o$; open bars). The summary data shows a partial blockade by picrotoxin and a complete blockade by bicuculline. Musc, muscimol; PTX, picrotoxin; Bic, bicuculline.

mimicked with depolarizing current injection, suggesting the involvement of voltage-gated $[Ca^{2+}]$ channels. This effect was not mimicked by baclofen, the GABA receptor antagonist. To date, the most direct evidence that intracellular free-Ca^{2+} levels are increased by synaptically-released GABA comes from primary culture studies of the rat hypothalamus, where bicuculline causes a further reduction of $[Ca^{2+}]_i$ in the presence of AP5 and CNQX.[45] A similar finding has been obtained in acute brain slices from neonatal rat cortex.[47]

Implications for developmental plasticity of inhibitory synapses

There are now several studies that demonstrate an influence of GABAergic transmission on the maturation of neuron structure or synaptogenesis.[13,23,35,36,39,70,73,74] For example, femtamolar GABA concentrations exert a tropic influence on embryonic spinal cord neurons,[6] and $GABA_A$ receptors regulate neurite growth and branching in embryonic hippocampal neurons.[5] Our previous studies in the gerbil LSO also support a role for glycinergic transmission during the maturation of dendritic branching, terminal arborizations within the LSO, and postsynaptic physiology.[4,31,55–57,59] Therefore, the ionic and metabolic changes that attend inhibitory synaptic transmission are of some importance during development.

It is conceivable that the present findings reflect a particular conformation of $GABA_A$ receptors. *In situ* hybridization and immunohistological studies demonstrate significant alterations in $GABA_A$ receptor subunit expression and composition during postnatal brain development, including in the IC.[18,32] Electrophysiological recordings in acutely dissociated hippocampal neurons have revealed a transiently-expressed GABA receptor that is sensitive to picrotoxin, but relatively insensitive to 100 μM bicuculline.[34] Moreover, ageing studies demonstrate that alterations of the GABAergic system continue into adulthood. These changes include a reduction in GABA-containing neurons and the release of GABA,[9] a reduction of $GABA_A$ receptor subunits and glutamic acid decarboxylase,[22] and an enhancement of benzodiazepine potency.[20]

Implication for normal function of the inferior colliculus

Although we have emphasized the developmental implications of the present findings, they may also hold implications for auditory processing in juvenile and adult animals. Recordings from cricket central auditory neurons clearly show the important kinetics of acoustically-evoked calcium signals.[65] Moreover, depolarizing inhibitory responses have been identified at GABAergic and glycinergic synapses in the developing and mature auditory brainstem.[19,25,28] Therefore, inhibitory transmission may contribute two general effects in IC neurons. When both inhibitory and excitatory synapses are co-active, as is often the case during acoustic stimulation,[10,14,43] the postsynaptic calcium levels are likely to be maintained at relatively low levels by the inhibitory synapses. However, when acoustic stimuli primarily recruit excitatory synaptic responses, then $[Ca^{2+}]_i$ would be expected to rise significantly. The *in vitro* paradigms employed in the present study (Figs 2, 3) suggest that this inhibitory mechanism plays a prominent role in the IC. In contrast, when synaptic activation is dominated by intense inhibitory activity, which may occur under specific stimulus conditions,[14,26] the inhibitory afferents may transiently elicit a membrane depolarization and an increase in postsynaptic free calcium (Figs 4–6). It is important to stress that the young age of tissue examined in our studies prevents us from drawing strong conclusions about adult properties.

We would speculate that these GABA-associated phenomena could play a role in auditory processing

of long-lasting sound stimuli. For example, we have recently shown that a period of strong, acoustically-evoked inhibition leads to enhanced sound-evoked excitation that persists after the inhibitory stimulus level has been decreased.[58,69] One hypothesis is that inhibitory activity transiently alters postsynaptic IC neuron excitability by means of calcium entry.

CONCLUSIONS

These results suggest the involvement of two mechanisms of inhibitory transmission in the IC during the developmental period examined. Inhibitory transmission can suppress synaptically-evoked rises in $[Ca^{2+}]_i$, yet a GABAergic mechanism is also able to mediate a rise in $[Ca^{2+}]_i$. Such long-lasting responses to inhibitory transmission are an interesting candidate for mediating trophic interactions during development,[31,59] and they may also have implications for acoustic stimulus processing in the IC.

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