Three GABA Receptor-Mediated Postsynaptic Potentials in Interneurons in the Rat Lateral Geniculate Nucleus

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Inhibition is crucial for the thalamus to relay sensory information from the periphery to the cortex and to participate in thalamocortical oscillations. However, the properties of inhibitory synaptic events in interneurons are poorly defined because in part of the technical difficulty of obtaining stable recording from these small cells. With the whole-cell recording technique, we obtained stable recordings from local interneurons in the lateral geniculate nucleus and studied their inhibitory synaptic properties. We found that interneurons expressed three different types of GABA receptors: bicuculline-sensitive GABA_A receptors, bicuculline-insensitive GABA_A receptors, and GABA_B receptors. The reversal potentials of GABA responses were estimated by polarizing the membrane potential. The GABA_A receptor-mediated responses had a reversal potential of approximately -82 mV, consistent with mediation via Cl[−] channels. The reversal potential for the GABA_B response was -97

mV, consistent with it being a K⁺ conductance. The roles of these GABA receptors in postsynaptic responses were also examined in interneurons. Optic tract stimulation evoked a disynaptic IPSP that was mediated by all three types of GABA receptors and depended on activation of geniculate interneurons. Stimulation of the thalamic reticular nucleus evoked an IPSP, which appeared to be mediated exclusively by bicuculline-sensitive GABA_A receptors and depended on the activation of reticular cells. The results indicate that geniculate interneurons form a complex neuronal circuitry with thalamocortical and reticular cells via feed-forward and feedback circuits, suggesting that they play a more important role in thalamic function than thought previously.

Key words: dendrite; cortex; visual cortex; thalamus; thalamic reticular nucleus; lateral posterior nucleus; superior colliculus; inhibitory circuits; oscillation; epilepsy; GABA_C receptor; retina

The thalamus is the primary structure that relays sensory information from the periphery to the cortex in mammals (Sherman and Koch, 1986; Sherman and Guillery, 1996). The thalamus is also involved in normal and abnormal synchronized neuronal activity (Steriade et al., 1993; von Krosigk et al., 1993; Huguenard and Prince, 1994b; Warren et al., 1994). These sensory and oscillatory functions are primarily mediated by the three basic types of thalamic neurons (Jones, 1985): thalamocortical cells, local interneurons, and cells in the thalamic reticular nucleus (TRN cells or cells in the equivalent perigeniculate nucleus). Thalamocortical cells are excitatory neurons, and they alone have axons that project to the cortex. In contrast, local interneurons and TRN cells are GABAergic cells, and their axons project locally within the thalamus to form complex inhibitory circuits. The inhibitory circuits can provide different forms of inhibition on thalamocortical cells, which are not only important for sculpting ascending sensory signals (Norton and Godwin, 1992; Soltesz and Crunelli, 1992) but also for promoting and synchronizing the thalamic oscillations (Steriade et al., 1993, 1996).

Of those of the two broad classes of inhibitory neurons in the thalamus, the properties of inhibitory connections and synapses made by TRN cells have been well studied (Ahlsén et al., 1985; Lo, 1985; Crunelli et al., 1988; Thomson, 1988; Paré et al., 1991;

Huguenard and Prince, 1994a; Warren et al., 1994; Bal et al., 1995; Ulrich and Huguenard, 1996; Sanchez-Vives et al., 1997). It has been demonstrated that in addition to projecting their axons to the thalamic relay nuclei, TRN cells also form mutual inhibitory connections within the TRN. The mutual inhibitory connections, like those from TRN to thalamocortical cells, are mediated by both GABA_A and GABA_B receptors (Ulrich and Huguenard, 1996; Kim et al., 1997; Sanchez-Vives et al., 1997), which are believed to be crucial for TRN cells to mold their output patterns (i.e., burst or tonic) and subsequently to determine the forms of inhibition imposed on thalamocortical cells (Huguenard and Prince, 1994a; Destexhe and Sejnowski, 1995; Kim et al., 1997). In contrast, the inhibitory connections to local interneurons have not been well studied because in part of the technical difficulty of obtaining stable recording from these small cells (Ahlsén et al., 1984). Furthermore, the composition of GABA receptors involved in the inhibitory synapses on interneurons remains unclear. The whole-cell recording technique makes small neurons more accessible for intracellular recording (Hamill et al., 1981). Using this technique, we routinely obtained stable recordings from geniculate interneurons that lasted 1–4 hr, allowing us to examine both electrophysiological and pharmacological properties of the GABAergic synapses formed on interneurons. The results provide a more detailed description of the local inhibitory circuits in the thalamus.

MATERIALS AND METHODS

Experiments were performed in thalamic slices from Wistar rats. These animals were postnatal 30–60 d old (100–280 gm), and no differences in electrophysiological properties of interneurons were found over this age range. The rats were deeply anesthetized by halothane and decapitated.

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Figure 1. A geniculate interneuron. *A*, Morphology of a reconstructed geniculate interneuron. *B*, Location of the cell in the LGN. Note that the coronal section contains no TRN. *vLGN*, Ventral LGN; *LP*, lateral posterior nucleus; *OT*, optic tract. *C*, Responses of the same interneuron to depolarizing and hyperpolarizing current pulses. The resting potential of this cell was -76 mV.

The brain was then quickly removed and placed into cold ($1-4^{\circ}\text{C}$) physiological solution containing (in mM): NaCl 125, KCl 2.5, NaH_2PO_4 1.25, NaHCO_3 25, MgCl_2 1, dextrose 25, and CaCl_2 2, at pH 7.4. The solution was continuously bubbled with 95% O_2 /5% CO_2 . Slices containing LGN, each 300–500 μm thick, were cut either coronally or parasagittally from the tissue blocks with a microslicer (Campden Instrument). These slices were kept in a warmed ($37.0 \pm 0.5^{\circ}\text{C}$), oxygenated physiological solution for ~ 1 hr before recordings were made. During the recordings, slices were submerged in a Plexiglas chamber and stabilized using a fine nylon net attached to a platinum ring. The chamber was perfused with warmed, oxygenated physiological solution, and the half-time for the bath solution exchange was ~ 6 sec. The temperature of the bath solution in the chamber was kept at $35.0 \pm 0.5^{\circ}\text{C}$. Agonists and antagonists were typically applied with the bath solution at 7–15 min intervals. Approximately 4–7 min was allowed to wash in and wash out the antagonists. Local application of glutamate within TRN was achieved by a brief application of pressure to the back of the pipette containing 250 μM glutamate in physiological solution (cf. Stuart and Sakmann, 1995). During this experiment, TRN was always on the downstream side of the bath solution flow, and a dye, pontamine sky blue, was included in the glutamate solution to make sure that glutamate did not spread into other nuclei.

Whole-cell recordings from interneurons were made either with the blind-patch technique (Blanton et al., 1989; Edwards et al., 1989) or with the aid of differential interference contrast optics (Stuart et al., 1993). Patch electrodes were made from borosilicate tubing, and their resistances were 5–9 M Ω with our intracellular solution. The standard intracellular solution was (in mM): potassium gluconate 115, HEPES 10, MgATP 2, Na_2ATP 2, GTP 0.3, and KCl 20, with 0.25% biocytin, at pH 7.3. An 11 mV liquid junction potential was subtracted from all membrane potentials. An Axoclamp-2B amplifier (Axon Instruments) was used to record voltage responses. The optic tract and TRN were stimulated by a concentric bipolar electrode (FHC Inc., Bowdoinham, ME) with single (200 μsec ; 15–115 μA ; 0.25 Hz) or short (three pulses at 100–400 Hz) trains of current pulses. The recording traces typically contained single sweeps except for those (see Figs. 6*A*, 7*A*) that had five superimposed sweeps. After recordings were made, slices were directly fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer, resected into 150- to 250- μm -thick sections, and histologically reacted for biocytin to recover the cell morphology (Horikawa and Armstrong, 1988). Cells were subsequently drawn at 100 $\times$ magnification with the aid

of a computerized reconstruction system (NeuroLucida 3.18). Chemicals were purchased from Research Biochemicals (Natick, MA).

RESULTS

Neuronal identification

We studied inhibitory receptors and IPSPs in 60 interneurons in the rat's LGN using the whole-cell recording technique (Fig. 1). After the whole-cell configuration was formed, interneurons were easily identified by their characteristic physiological properties, such as high input resistance and long membrane time constant (Fig. 1*C*) (Leresche et al., 1991; Pape et al., 1994; Williams et al., 1996; Zhu and Uhlrich, 1997; Zhu et al., 1999b). The morphology of all 60 cells was recovered, confirming that the recordings were indeed obtained from interneurons (Grossman et al., 1973; Ohara et al., 1983; Ottersen and Storm-Mathisen, 1984; Webster and Rowe, 1984; Gabbott et al., 1985, 1986). Unlike thalamocortical cells, which have a relatively large soma and a multipolar appearance (Zhu and Uhlrich, 1997; Zhu et al., 1999a), interneurons typically had a bipolar appearance with two dendrites arising from the opposite sides of the soma (Fig. 1*A*). The two long complex dendrites, beaded and having intricate branching patterns, encompassed a relatively large area within the LGN (Fig. 1*B*). Seven interneurons, however, had three complex dendrites arising from the soma (data not shown) (but see Zhu et al., 1999a). In most interneurons, a putative axon, with relatively fine caliber and frequent en passant varicosities, could be identified. The axon typically originated from the soma or a proximal dendrite and ramified locally within the LGN. The axonal and dendritic arbors tended to be spatially offset from each other, with the axon arbor occupying a relatively smaller area near the soma.

GABA_A and GABA_B receptors in interneurons

We first studied the responses of interneurons to GABA. A brief application (5–7 sec) of 500 μM GABA with the bath solution

Figure 2. Responses of a rat geniculate interneuron to a brief bath application of GABA. *A*, GABA (500 μ M; horizontal bar) induced a prolonged response in a geniculate interneuron, which appeared to consist of two components. *B*, Plotting the peak amplitudes of the early response against the membrane potential revealed its reversal potential to be -82.5 mV. *C*, Plotting the amplitudes of the response at 50 sec after the GABA application against the membrane potential revealed its reversal potential to be -99.0 mV. The data points in *B* and *C* were fitted by linear lines. The resting potential of this cell was -64 mV.

induced a prominent hyperpolarization at the resting or a slightly depolarized membrane potential (up to -55 mV; $n = 33$; Fig. 2). The hyperpolarization lasted 50–80 sec and appeared to contain two components. The early component had a large amplitude (up to 25 mV) and fast time course and was accompanied by a large decrease in input resistance. In contrast, the later one had a small amplitude (up to 8 mV) and slow time course and was marked by a small reduction in input resistance (Fig. 2*A*). Plotting the peak amplitude of the early polarization against the holding potential gave the reversal potential of the early response to be -81.5 ± 3.2 mV ($n = 9$; Fig. 2*B*), whereas the reversal potential of the later response was -97.2 ± 2.3 mV ($n = 9$; Fig. 2*C*). The result suggests that the GABA responses in interneurons are mediated by at least two different conductances.

To examine whether the two GABA responses were mediated by GABA_A and GABA_B receptors, respectively, we performed a series of pharmacological experiments. The cells were initially hyperpolarized to approximately -90 mV to reverse the early GABA response (Fig. 3*A*). Adding 20 μ M bicuculline, a GABA_A receptor blocker (Sivilotti and Nistri, 1991; Thompson, 1994), suppressed the early depolarization, whereas the later hyperpolarization was little affected ($n = 5$; Fig. 3*B*). Adding 1 mM saclofen, a GABA_B receptor blocker (Sivilotti and Nistri, 1991; Thompson, 1994), completely blocked the later hyperpolarization ($n = 5$; Fig. 3*D*). The results indicate that both GABA_A and GABA_B receptors are involved in the GABA responses in interneurons.

As shown in Figure 3, 20 μ M bicuculline only partially blocked the early depolarizing response (by $66.4 \pm 7.3\%$; $n = 5$). Increasing bicuculline from 20 to 200 μ M had no additional effect on the

residual depolarization ($n = 3$; Fig. 3*C*), indicating the existence of a bicuculline-resistant response. The reversal potential of the residual response was -82.9 ± 3.4 mV ($n = 7$), similar to that of the bicuculline-sensitive response. This response was also insensitive to 10 μ M strychnine ($n = 3$; data not shown), a glycine receptor blocker (Gillette and Dacheux, 1995). We speculated that it might be mediated by bicuculline-insensitive GABA_A receptors and tested this hypothesis with picrotoxin (PTX), a Cl[−] channel blocker (Akaike et al., 1985), which blocks both bicuculline-sensitive and -insensitive GABA_A responses (Lukasiewicz et al., 1994; Qian and Dowling, 1994). Bicuculline and saclofen were used to isolate the bicuculline-insensitive response (Fig. 4*A,B*). Adding 10 μ M PTX completely blocked the response ($n = 5$; Fig. 4*C*). The result suggests that bicuculline-insensitive GABA_A receptors are also present in interneurons.

Bicuculline-insensitive GABA_A receptors have been extensively studied in the retina (Feigenspan et al., 1993; Qian and Dowling, 1993; Lukasiewicz et al., 1994; Matthews et al., 1994). These studies have demonstrated that a conformationally restricted GABA analog *cis*-4-aminocrotonic acid (CACA) is a potent agonist for the bicuculline-insensitive GABA_A receptors. To confirm that geniculate interneurons express two types of GABA_A receptors, we also used CACA as the receptor agonist. Briefly applying 250 μ M CACA induced a hyperpolarization at the resting or a slightly depolarized potential (up to -55 mV; Fig. 5*A*). The response lasted ~ 40 –50 sec and had a reversal potential of -82.2 ± 2.7 mV ($n = 5$).

CACA can selectively activate bicuculline-insensitive GABA_A receptors in some retinal neurons but not in other ones (Lukasiewicz et al., 1994; Gillette and Dacheux, 1995). We exam-

Figure 3. Responses of a rat geniculate interneuron to a brief bath application of GABA. *A*, A brief bath application of 500 μ M GABA induced a depolarization and a hyperpolarization in an interneuron. *B*, The depolarization was suppressed by bath application of 20 μ M bicuculline. *C*, Increasing bicuculline to 200 μ M had little additional effect on the residual depolarization. *D*, Adding 1 mM saclofen in the bath solution completely blocked the hyperpolarization. *E*, The response recovered after washing in the normal bath solution. The resting membrane potential of this cell was -61 mV, and the cell was held at -90 mV during the experiment.

ined whether CACA can be used to activate bicuculline-insensitive GABA_A receptors in interneurons selectively. Bath application of 20 μ M bicuculline blocked $66.0 \pm 10.0\%$ of the CACA-induced hyperpolarization ($n = 5$; Fig. 5*A,B*), suggesting that CACA activated both bicuculline-sensitive and -insensitive GABA_A receptors in interneurons. Adding bicuculline up to 200 μ M had no additional effect ($n = 3$). The residual response reversed at -83.5 ± 3.0 mV ($n = 3$; data not shown) and was completely blocked by bath application of 10 μ M PTX ($n = 5$; Fig. 5*C*), indicating a Cl⁻-dependent mechanism.

Together, the results suggest that the GABA-induced responses in interneurons are mediated by three types of GABA receptors: bicuculline-sensitive GABA_A receptors, bicuculline-insensitive GABA_A receptors, and GABA_B receptors.

Interneuron-mediated inhibition in interneurons

To examine whether all three types of GABA receptors are involved in generating IPSPs in these interneurons, we studied the synaptic responses of these interneurons to stimulation of the optic tract and TRN.

We first investigated the responses induced by the optic tract stimulation, using only the coronal slices that did not contain TRN (Fig. 1*B*). A single electric shock induced a long-lasting EPSP in interneurons at the resting membrane potential, which could reach threshold and elicit action potentials (Fig. 6*A*). No

obvious IPSP was detected in this condition, presumably because of the presence of the prolonged EPSP. However, when cells were depolarized to approximately -5 mV, a prominent IPSP (peak amplitude, 16.6 ± 2.4 mV; $n = 7$) was revealed (Fig. 6*B*). The IPSP lasted 293 ± 19 msec and consisted of a large, fast hyperpolarization and a small, slow tail. The fast hyperpolarization was primarily suppressed by bath application of 20 μ M bicuculline, leaving a small (1.4 ± 0.3 mV) but prolonged (285 ± 21 msec) hyperpolarization ($n = 7$; Fig. 6*C*). Adding 1 mM saclofen selectively blocked the later portion of the hyperpolarization ($n = 7$; Fig. 6*D*). Although the residual hyperpolarization (0.9 ± 0.2 mV; 153 ± 14 msec) was little affected by additional bicuculline (up to 100 μ M; $n = 2$; data not shown), it was completely abolished by 10 μ M PTX ($n = 7$; Fig. 6*E*). Therefore, these results suggest that all three types of GABA receptors contribute to the optic tract-evoked IPSP in interneurons.

We then tested whether the optic tract-elicited IPSP was dependent on the activation of interneurons. After the IPSP was induced by the optic tract stimulation (Fig. 7*A,B*), 20 μ M 6,7-dinitroquinoxaline-2,3-dione (DNQX), a kainate and AMPA receptor blocker, and 50 μ M D(-)-2-amino-5-phosphonopentanoic acid (D-AP-5), an NMDA receptor blocker, were applied with the bath solution. DNQX and D-AP-5 reversibly blocked the IPSP ($n = 4$; Fig. 7*C,D*). The results together suggest that the IPSP is

Figure 4. Responses of a rat geniculate interneuron to a brief bath application of GABA. *A*, A brief bath application of 500 μM GABA induced a hyperpolarization in an interneuron. *B*, The hyperpolarization was partially blocked by bath application of 20 μM bicuculline and 2 mM saclofen. *C*, The residual response was completely blocked by adding 10 μM PTX in the bath solution. *D*, The response partially recovered after washing in the normal bath solution. The resting membrane potential of this cell was -63 mV.

a disynaptic potential, resulting from the activation of interneurons. The results also indicate that the IPSP is mediated by three different types of GABA receptors.

TRN cell-mediated inhibition in interneurons

The TRN cell-mediated inhibition was also studied. We sectioned the slices parasagittally so that both the LGN and the visual sector of the TRN could be included in the same slice (Fig. 8*B*). We found no difference in electrophysiological properties between the interneurons recorded from coronally cut slices and those from sagittally cut ones (Figs. 1*C*, 8*C*). The morphology of these cells was also similar (Figs. 1*A*, 8*A*).

Because stimulating the TRN may excite interneurons by activating excitatory thalamocortical and corticothalamic fibers that pass through the nucleus, 20 μM DNQX and 50 μM D-AP-5 were included in the bath solution during the following experiments. Single electric shock in the TRN induced an IPSP in interneurons ($n = 5$; Fig. 9*A*). It had a duration of 134 ± 15 msec and an amplitude of 0.43 ± 0.16 mV. The duration and amplitude of the IPSP increased to 218 ± 23 msec and 1.74 ± 0.60 mV, respectively, when a train of three shocks was applied (Fig. 9*B*). Application of 20 μM bicuculline reversibly blocked the IPSP ($n = 5$; Fig. 9*C,D*), suggesting that the IPSP might be mediated exclusively by bicuculline-sensitive GABA_A receptors.

To eliminate the possibility that the IPSP might result from the activation of inhibitory fibers bypassing the TRN, we also used another approach, local application of glutamate within the TRN, to activate TRN cells. Application of glutamate in the TRN induced a hyperpolarization in interneurons (Fig. 10*A*). The hyperpolarization was reversibly blocked by 20 μM bicuculline ($n = 4$; Fig. 10*B,C*). The result confirms that TRN cells form inhibitory synapses on interneurons and that the inhibition may be mediated exclusively by bicuculline-sensitive GABA_A receptors.

DISCUSSION

In this study, we demonstrated that three different types of GABA receptors (bicuculline-sensitive GABA_A receptors, bicuculline-insensitive GABA_A receptors, and GABA_B receptors) are present in rat geniculate interneurons. All three types of GABA receptors are involved in the interneuron-mediated inhibition, whereas only bicuculline-sensitive GABA_A receptors appear to be involved in the TRN cell-mediated inhibition.

Three types of GABA receptors in interneurons

A recent study reported that all interneurons expressed GABA_A receptors and that only a small fraction of them also had GABA_B receptors (Williams et al., 1996). Another study (Pape and McCormick, 1995), however, suggested that both GABA_A and GABA_B receptors are present in interneurons. Our results are consistent with the latter findings in that although the GABA_B receptor-mediated response was typically very small, GABA responses were always mediated by both GABA_A and GABA_B receptors in geniculate interneurons.

We found that the GABA_A receptor-mediated response, induced by both GABA and CACA, had a reversal potential of approximately -82 mV, which was comparable with that in thalamocortical cells (Huguenard and Prince, 1994b; Sanchez-Vives and McCormick, 1997; Ulrich and Huguenard, 1997). This reversal potential is more negative than the calculated Cl^- equilibrium potential (-44 mV), suggesting that an active Cl^- extrusion mechanism may also exist in interneurons (Ulrich and Huguenard, 1997). In addition, in this study, we showed that the reversal potential of the GABA_B response was approximately -97 mV, congruent with it being mediated by a K^+ conductance (the calculated K^+ equilibrium potential is -100 mV).

The existence of bicuculline-insensitive GABA_A receptors was first confirmed in the retina (Lukasiewicz et al., 1994; Qian and

Figure 5. Responses of a rat geniculate interneuron to a brief bath application of CACA. *A*, A brief bath application of 250 μ M CACA induced a hyperpolarization in an interneuron. *B*, The hyperpolarization was partially blocked by bath application of 20 μ M bicuculline. *C*, The residual response was completely blocked by adding 10 μ M PTX in the bath solution. *D*, The response recovered after washing in the normal bath solution. The resting membrane potential of this cell was -69 mV.

Dowling, 1994). The receptors, also named GABA_C receptors, were found to be coupled with Cl[−] channels and sensitive to PTX (but see Feigenspan et al., 1993). There is evidence that the receptors might also exist in the other nuclei in the CNS (Drew et al., 1984; Sivilotti and Nistri, 1989). A recent molecular biological study has suggested that this type of receptor protein is expressed in the LGN, although restricted to a subgroup of geniculate neurons (Wegelius et al., 1998). Because thalamocortical cells in the LGN do not have bicuculline-resistant GABA_A conductances (Crunelli et al., 1988; Bal et al., 1995), by default, the receptors must exist in the other group of cells in the LGN, namely, geniculate interneurons. Our finding that there is a bicuculline-insensitive GABA_A response in geniculate interneurons is consistent with this notion. This is the first direct evidence that bicuculline-insensitive GABA_A receptors mediate synaptic potentials in another region of the CNS besides the retina.

Local inhibitory circuits in the thalamus

We demonstrated that TRN cells inhibit interneurons in rats. This is in good agreement with our previous *in vivo* study in cats (Ahlsén et al., 1985). However, whether the similar connections exist in other mammals, such as ferrets, remains elusive (Bal et al., 1995; Sanchez-Vives and McCormick, 1997). Compared with the TRN cell-mediated IPSP in thalamocortical cells (Huguenard and Prince, 1994a), the TRN cell-mediated IPSP in interneurons had a relatively small amplitude. This may be because only a limited number of TRN cell–interneuron connections were pre-

served in the slices. It is also possible that TRN cells form fewer synapses on interneurons than on thalamocortical cells, as suggested by a recent anatomical study (Liu et al., 1995). Interestingly, this TRN cell-mediated inhibition appeared to be mediated by GABA_A receptors only. However, we cannot eliminate the possibility that GABA_B receptors may also be activated in certain conditions [e.g., during the very strong activation of TRN cells and the accumulation of GABA at the synapses (Otis and Mody, 1992; Isaacson et al., 1993; Huguenard and Prince, 1994a; Kim et al., 1997)].

Our previous *in vivo* study suggested that the cortical stimulation can induce an IPSP in geniculate interneurons that is mediated by the inhibitory circuit from TRN cells (Ahlsén et al., 1985). However, whether there was an interneuron-mediated inhibition in interneurons is still unclear because the optic tract stimulation-evoked IPSP may result from the activation of the recurrent inhibitory circuit alone (Ahlsén et al., 1985). Using the *in vitro* preparation, we could cut LGN slices that contained no TRN. Stimulation of the optic tract in these slices evoked a prominent IPSP in all geniculate interneurons recorded, suggesting the existence of an interneuron-mediated inhibition. This result was confirmed by bath application of DNQX and D-AP-5, which block excitatory retinogeniculate transmission (Scharfman et al., 1990; Zhu et al., 1999a). As expected, blocking the excitation of interneurons eliminated the IPSP. This result is in line with the anatomical finding that interneurons may form mutual inhibitory synaptic connections (Famiglietti, 1970; Wong, 1970; Lieberman and Webster, 1974; Pasik et al., 1976).

Our pharmacological experiments suggest that although three types of GABA receptors are involved in the interneuron-mediated inhibition, only bicuculline-sensitive GABA_A receptors may be involved in the TRN cell-mediated inhibition. From these experiments, together with the knowledge from previous *in vivo* (e.g., Ahlsén et al., 1985; Lo, 1985; Paré et al., 1991; Zhu and Lo, 1997, 1998) and *in vitro* studies (e.g., Crunelli et al., 1988; Thomson, 1988; Lo and Sherman, 1994; Bal et al., 1995; Ulrich and Huguenard, 1996), a more detailed picture of thalamic local inhibitory circuits has emerged. Figure 11 is a diagram for the thalamic local circuitry, which is simplified to emphasize its major characteristics. TRN cells receive excitatory inputs from thalamocortical cells and give feedback GABA_A and GABA_B receptor-mediated inhibition to thalamocortical cells. TRN cells also form mutual inhibitory connections mediated by both GABA_A and GABA_B receptors. In addition, TRN cells synapse on interneurons and inhibit them via GABA_A receptors. As with thalamocortical cells, interneurons also receive excitatory inputs directly from ganglion cells in the retina. Besides forming mutual inhibitory connections via GABA_A and GABA_B receptors, interneurons also provide feed-forward inhibition to thalamocortical cells, which is mediated by both GABA_A and GABA_B receptors. It is still unclear whether interneurons receive synaptic inputs from thalamocortical cells, but the connections may be rare if they do exist (Friedlander et al., 1981; Montero, 1991).

Functional considerations

Three types of GABA receptors and two inhibitory pathways allow more controlling of the excitation and firing properties of interneurons (Connors et al., 1988; Connors, 1992; Ferster and Jagadeesh, 1992; Mody et al., 1994) and subsequently of the inhibition patterns imposed onto thalamocortical cells (Huguenard and Prince, 1994a; Destexhe and Sejnowski, 1995; Kim et

Figure 6. Responses of a rat geniculate interneuron to stimulation of the optic tract. *A*, Single electric shock induced a prolonged EPSP in an interneuron, which could reach threshold and elicit action potentials. *B*, Depolarizing the cell to -5 mV revealed a prominent IPSP. *C*, A substantial part of the IPSP was blocked by the bath application of $20 \mu\text{M}$ bicuculline. *D*, The bicuculline-resistant IPSP was partially blocked by adding 1 mM saclofen to the bath solution. *E*, The residual IPSP was blocked by $10 \mu\text{M}$ PTX. *F*, The response recovered after washing in the normal bath solution. The resting membrane potential of this cell was -74 mV .

Figure 7. Responses of a rat geniculate interneuron to stimulation of the optic tract. *A*, Single electric shock induced a prolonged EPSP in an interneuron, which could reach threshold and elicit action potentials. *B*, Depolarizing the cell to -5 mV revealed a prominent IPSP. *C*, The IPSP was completely blocked by the bath application of $20 \mu\text{M}$ DNQX and $50 \mu\text{M}$ D-AP-5. *D*, The response recovered after washing in the normal bath solution. The resting membrane potential of this cell was -74 mV .

al., 1997). For example, GABA_A receptor-mediated IPSPs dominated in interneurons and had a quick time course. They may provide robust suppression of the spiking activity with fine temporal control. GABA_B receptor-mediated IPSPs were very small and had a slow time course. They may elevate the threshold for spiking activity for a relatively long period. In addition, the feed-forward and feedback inhibitory circuits may affect the synaptic integration and spiking activity differently because the feed-forward synapses possess both GABA_A and GABA_B receptors whereas the feedback synapses have only GABA_A receptors. Furthermore, expressing two types of GABA_A receptors in in-

terneurons may also be of functional importance because preliminary evidence suggests that bicuculline-sensitive and -insensitive GABA_A receptors might be located primarily in the soma/proximal dendrites and distal dendrites, respectively (J. J. Zhu and F.-S. Lo, unpublished observations). In geniculate interneurons, both the axon and dendrites can release neurotransmitter (Ralston, 1971; Cox et al., 1998). Thus, activation of GABA_A receptors may regulate the dendritic release sites by reducing both dendritic excitation and calcium influx via bicuculline-insensitive GABA_A receptors (Matthews et al., 1994; see also Kim et al., 1995; Chen and Lambert, 1997; Larkum et al., 1999) and may

Figure 8. A geniculate interneuron. *A*, Morphology of a reconstructed geniculate interneuron. *B*, Location of the cell in the LGN. Note that the parasagittal section contains both the LGN and TRN. *MG*, Medial geniculate nucleus. *C*, Responses of the same interneuron to depolarizing and hyperpolarizing current pulses. The resting potential of this cell was -67 mV.

Figure 9. Responses of a rat geniculate interneuron to stimulation of the TRN. *A*, Single electric shock in the TRN induced a small IPSP in an interneuron. *B*, A train of three shocks at 250 Hz induced a larger IPSP in the same interneuron. *C*, The IPSP was completely blocked by the bath application of $20 \mu\text{M}$ bicuculline. *D*, The response recovered after washing in the normal bath solution. Note that $20 \mu\text{M}$ DNQX and $50 \mu\text{M}$ D-AP-5 were included in the bath solution during the experiment. The resting membrane potential of this cell was -66 mV.

modulate the axonal release sites by suppressing the somatic excitation via bicuculline-sensitive GABA_A receptors.

In addition to relaying the sensory information from the periphery to the cortex, the thalamus is also involved in the normal and abnormal oscillations (Steriade et al., 1993; von Krosigk et al., 1993; Huguenard and Prince, 1994b; Warren et al., 1994). Like thalamocortical cells and TRN cells, thalamic interneurons can generate an intrinsic oscillation (Zhu et al., 1999a) and partici-

pate in the synchronized thalamic oscillations (Deschênes et al., 1984; Steriade and Deschênes, 1984). In the normal condition, interneurons may be involved in a 7–12 Hz oscillation (Deschênes et al., 1984; Zhu et al., 1999a). The mutual inhibitory connections between interneurons, which are mediated predominantly by GABA_A receptors, are perfect for promoting and synchronizing this rhythm. However, if the GABA_A inhibition is disabled and GABA_B inhibition becomes dominant, the oscillation may trans-

Figure 10. Responses of a rat geniculate interneuron to local application of glutamate in the TRN. *A*, Local application of 250 μ M glutamate in the TRN induced a small hyperpolarization in an interneuron. *B*, The hyperpolarization was completely blocked by the bath application of 20 μ M bicuculline. *C*, The response recovered after washing in the normal bath solution. The resting membrane potential of this cell was -64 mV.

Figure 11. Inhibitory circuits in the thalamus.

form to the spike-and-wave activity (Bal et al., 1995; Sanchez-Vives and McCormick, 1997), an epileptic type of oscillation that has been observed in interneurons *in vivo* (Steriade and Deschênes, 1984). The functional meaning of the TRN cell-mediated inhibition in the thalamic oscillations is less obvious. However, the inhibition may be crucial for synchronizing the oscillations because its relatively small amplitude and brief time course appear to be ideal for interneurons to shift the oscillatory phase but not the oscillatory rhythm. Further study is necessary to determine the exact roles of individual cell- and receptor-

mediated inhibitions in interneurons during these thalamic oscillations.

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