GABA_B-Receptor-Mediated Inhibition in Developing Mouse Ventral Posterior Thalamic Nucleus

RICHARD A. WARREN,1 PEYMAN GOLSHANI,2 AND EDWARD G. JONES2
1Centre de Recherche Fernand-Seguin, Montreal, Quebec H1N 3V2, Canada; and 2Department of Anatomy and Neurobiology, University of California, Irvine, California 92697


Inhibitory postsynaptic potentials (IPSPs) generated by activation of the thalamic reticular nucleus (RTN) were recorded in neurons of the ventral posterior nucleus (VP) in vitro in slices from mice aged postnatal day (P) 1–P17. An early IPSP peaking 41 ± 2.5 (SE) ms after electrical stimulation of the internal capsule or RTN was found in 96% of VP neurons. This early IPSP was blocked by bicuculline, showing its dependence on γ-aminobutyric acid-A (GABA_A) receptors. A late IPSP peaking 357 ± 27 ms after the stimulus was observed in 22% of VP neurons in control medium but was uncovered in 38% of neurons when bicuculline was added. The late IPSP was blocked by addition of a GABA_B antagonist, 2-hydroxysaclofen, to the medium (Huguenard and Prince 1994a; Ulrich and Huguenard 1996) and 8 days old (Huguenard and Prince 1994a; Ulrich and Huguenard 1996) and under certain conditions in carnivores (Bal et al. 1995a; von Krosigk et al. 1993). It was not possible to demonstrate GABA_B-mediated inhibition in developing mouse ventral posterior thalamic nucleus.

INTRODUCTION

Low-frequency oscillations in the interconnected network of thalamic and cortical neurons are accompaniments of changes in conscious state (Steriade et al. 1993). Similar oscillations, characterized by 3-Hz spike and wave discharges in the cortical electroencephalogram (EEG), are accompaniments of petit mal epilepsy (Aicardi 1994). Spindlerike oscillations and spike and wave discharges have their basis in oscillations of interconnected thalamic relay neurons and GABAergic neurons of the reticular nucleus (Steriade et al. 1993). These depend in turn on generation of low-threshold calcium spikes that lead to recurrent bursts of action potentials as relay cells recover from γ-aminobutyric acid (GABA)-imposed inhibition (Bal et al. 1995a,b; Huguenard and Prince 1994a; Warren et al. 1994). Drugs such as ethosuximide, which are effective in reducing the incidence of absence seizures, interfere with the cycle of recurrent inhibition and rebound oscillation (Coulter et al. 1989; Huguenard and Prince 1994a).

Slow-wave sleep is typically absent in rodents and notably reduced in other mammalian species during the early postnatal period (Jouvet-Mounier et al. 1970) and absence seizures or seizure-like activity do not occur in human infants (Aicardi 1994) or in infant animal models (Marescaux et al. 1992b). This may be related to immaturity of the membrane and synaptic properties of thalamic relay and reticular neurons during early postnatal life; in mice these only reach the adult state and permit full development of thalamic oscillations toward the end of the second postnatal week (Warren and Jones 1997).

Generation of rebound bursts in relay neurons is largely dependent on inhibition by the reticular nucleus through fast-acting, ionotropic GABA_A receptors (Bal et al. 1995a; Huguenard and Prince 1994a; Warren et al. 1994). The role of GABA_B receptors may be more limited normally because GABA_B antagonists reportedly do not affect intrathalamic oscillations in vitro (Bal et al. 1995a; Warren et al. 1994).

Nevertheless, GABA_B receptor binding can be demonstrated in mouse thalamus in early postnatal life (e.g., Lin et al. 1993), and typical, GABA_B-mediated slow inhibitory postsynaptic potentials (IPSPs) and presynaptic GABA_B effects occur in thalamic neurons of rats aged postnatal day (P) ≥ 8 days old (Huguenard and Prince 1994a; Ulrich and Huguenard 1996) and under certain conditions in carnivores (Bal et al. 1995a; von Krosigk et al. 1993). It was not possible to demonstrate GABA_B-mediated IPSPs in earlier studies in mice (Warren and Jones 1997; Warren et al. 1994). In the present study we report their presence in ventral posterior nucleus (VP) neurons from birth.

METHODS

The methods have been previously described (Warren and Jones 1997; Warren et al. 1994). Thalamocortical slices 400 μm thick were prepared from ICR mice [postnatal day (P) 1–P17] and continuously perfused at room temperature (22–25°C) with artificial cerebrospinal fluid (ACSF) containing (in mM) 126 NaCl, 3 KCl, 1.25 NaH₂PO₄, 1.3 MgSO₄, 2.5 CaCl₂, 26 NaHCO₃, and 10 dextrose, pH 7.4 when bubbled with 95% O₂-5% CO₂. In 2 of 22 experiments, the ACSF contained 5 or 10 μM bicuculline methiodine (BMI) or bicuculline methchloride (BMC) throughout the experiment. Whole cell recordings were obtained with pipettes filled with (in mM) 140 potassium gluconate, 2 MgCl₂, 0.1 CaCl₂, 1.1 ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetie acid-KOH, 10 N-2-hydroxyethylpiperazine-N'-2-ethanesulfo acid, and 2 K₂ATP, pH adjusted to 7.3 ± 0.5 (SE) with KOH. Synaptic responses were evoked by cathodal stimulation consisting of 0.1-ms voltage pulses delivered at 0.05–1.0 Hz to the internal capsule or the thalamic reticular nucleus (RTN) through a monopolar tungsten electrode. A 10-mV junction potential was subtracted from all membrane potential measurements (Huguenard...
and Prince 1994a; Spigelman et al. 1992). Results are presented as means ± SE.

RESULTS

IPSPs evoked by electrical stimulation were recorded in 58 VP neurons from P1 to P17. All had resting membrane potentials negative to −50 mV and overshooting action potentials.

An early IPSP peaking 41 ± 2.5 ms (SE; range 18–93 ms, n = 49) after the stimulus was observed in 52 of 54 (96%) neurons recorded in slices perfused with normal ACSF. This early IPSP was reversibly blocked by the addition of 5–10 μM BMI or BMC in 37 neurons and was absent in 4 neurons recorded in slices perfused with ACSF containing BMI or BMC. The reversal potential of the early GABA<sub>A</sub> IPSP, measured in 46 neurons, became more negative with age (r<sup>2</sup> = 0.333, P < 0.0001), as previously described (Warren and Jones 1997).

A late IPSP with a peak at 357 ± 27 ms (range 200–697 ms, n = 24) after the stimulus was observed in 25 of 53 (47%) neurons. This late IPSP was observed in only 11 of 49 (22%) neurons recorded in control ACSF, but was uncovered in 10 of 26 (38%) neurons when BMI or BMC was added to the ACSF and was obtained in all 4 (100%) neurons recorded in slices perfused with ACSF containing BMI or BMC. This late IPSP was found in neurons from P1 to P17 and was abolished by addition of 100–200 μM 2-hydroxysaclofen (2-OHS, n = 7), indicating its dependence on GABA<sub>B</sub> receptors. The reversal potential of the late IPSP was measured only in neurons in which the peak of the response could be isolated, thus avoiding contamination by the GABA<sub>A</sub> IPSP. The reversal potential was −98 ± 1.3 mV (range −106 to −89 mV), on average 14 mV negative to the early component. As measured in 13 neurons, it showed no apparent relationship with postnatal age (r<sup>2</sup> = 0.155, P = 0.183).

At all ages, both GABA<sub>A</sub> and GABA<sub>B</sub> IPSPs were hyperpolarizing at resting membrane potential. In some neurons, the inhibitory response appeared monophasic in control ACSF (Fig. 1A1) and a late IPSP could only be detected when BMI was added to the bathing medium, revealing a long-duration late IPSP that peaked almost 700 ms after the stimulus and 650 ms after the peak of the early component (Fig. 1A2). The GABA<sub>B</sub> nature of the late IPSP was confirmed by its abolition in the presence of 2-OHS. In Fig. 1A2, the late IPSP became apparent only during the recovery phase of the early IPSP (Fig. 1A2, vertical dotted line). Putative GABA<sub>B</sub> IPSPs were also observed at P1 in control ACSF. In some neurons, in control ACSF, the earlier...
GABA_\text{A}_\text{b} response was lacking (Fig. 1A3), showing that GABA_\text{A}_\text{h} receptor can be activated under normal conditions. In other neurons, in control ACSF, the two IPSPs were seen and there was a clear distinction between the early and late IPSPs which could be recognized by the presence of a hump occurring between them. In these cases, each component could be individually reduced with the use of specific pharmacological agents (Fig. 1B) and the early IPSP visibly reversed before the late IPSP as the membrane was hyperpolarized (Fig. 1C).

The late IPSP could not be detected in the response as a function of membrane potential curve of certain VP neurons; in others, including at older ages, a late IPSP could only be uncovered by blocking the GABA_\text{A}_\text{h} response. Figure 1D shows a P14 neuron that displayed recurrent IPSPs in response to single-shock stimulation of the internal capsule (Fig. 1D1). There was a large early IPSP but no obvious late component (Fig. 1D2, CONTROL). The late IPSP may have been obscured by activation of the low-threshold calcium spike. Addition of BMI uncovered a large excitatory postsynaptic potential that was blocked by 20 \mu M 6-cyano-7-nitroquinolinaxine-2,3-dione and 50 \mu M D-2-amino-5-phosphonovaleric acid and revealed a small, late IPSP that was blocked by the addition of 2-OHS. Onset of the late GABA_\text{B} IPSP coincided with the peak of the earlier GABA_\text{A} IPSP and its peak occurred during the late recovery phase of the GABA_\text{A} IPSP, during which the low-threshold calcium spike was apparently activated (Fig. 1D3, vertical dotted lines).

The reversal potential of the GABA_\text{B} IPSP did not change during postnatal development, in contrast to the GABA_\text{A} IPSP. The most striking change was a shortening of the latency between stimulus and peak of the GABA_\text{A} response (Fig. 2A). A change of several hundred milliseconds was not paralleled by a change of comparable magnitude in the GABA_\text{A} response (Fig. 2B). The latency-to-peak of the GABA_\text{B} response was strongly correlated with postnatal age, explaining almost 75% of its variation. Less than 10% of the variation in the latency-to-peak of the GABA_\text{A} response could be explained by postnatal age.

**DISCUSSION**

GABA_\text{B} and GABA_\text{A} IPSPs are present in mouse VP neurons from P1, showing that functional postsynaptic receptors of both types are present in the thalamus from birth. In other brain regions GABA_\text{B} postsynaptic responses were not found before the end of the first postnatal week (Gaiarsa et al. 1995; Luhmann and Prince 1991). GABA_\text{B} IPSPs were not previously detected (Warren and Jones 1997; Warren et al. 1994), probably because of masking by the large GABA_\text{A} IPSPs or because of washing out of its second-messenger system constituents. We observed an increased probability of evoking a GABA_\text{B} IPSP when GABA_\text{A} -mediated inhibition was blocked by adding bicuculline to the perfusing medium. The blockade of GABA_\text{A} receptors produces much larger bursts in RTN neurons through disinhibition (e.g., Bal et al. 1995b; Huguenard and Prince 1994b; Warren and Jones 1997). This would result in the release of a larger amount of GABA, which may be necessary to evoke a GABA_\text{B} IPSP (Destexhe and Sejnowski 1995).

GABA_\text{A} and GABA_\text{B} receptor functions are differentially regulated during postnatal development. The most significant change in the GABA_\text{A} IPSP was hyperpolarization of its reversal potential; this could be attributed to changes in Cl^- gradients occurring during the first postnatal weeks (Zhang et al. 1991). There was no change in the reversal potential of the GABA_\text{B} IPSP, but there was a large change in the kinetics of the response. This cannot be attributed to alterations in axonal conductivity or neurotransmitter release because these should have affected GABA_\text{A} IPSPs as well. Postsynaptic changes in membrane input resistance and time.
constant should also have affected GABA_A and GABA_B IPSPs equally. The changes in kinetics are, therefore, likely to be attributable to changes in transduction of synaptic signals through guanosine 5’-triphosphate-binding proteins and may reflect speeding up of the steps between binding of GABA to the receptor and gating of the K+ channel. This could be achieved through an increase in GABA concentration at the GABA_A receptor site due to developmental changes in uptake mechanisms or to an increased density of terminals, reducing the delay needed for the buildup of active G protein to reach a level sufficient to activate the K+ channel (Destexhe and Sejnowski 1995).

In the preparation used, intrathalamic oscillations are readily generated through synaptic interactions between RTN and VP neurons (Warren et al. 1994). In this and similar preparations from other species (Bal et al. 1995a), GABA_A antagonists produce no change in oscillations, suggesting that GABA_A-mediated-inhibition has a limited role in normal spindle formation. Recent findings, however, suggest an active role in slower thalamic oscillations similar to those observed in absence seizures (Bal et al. 1995a; Marescaux et al. 1992a), which generally do not occur in infants. The present study shows that functional GABA_A receptors are present from birth and therefore could participate in genesis of absence attacks.

The presence of GABA_B IPSPs in the thalamus during the early postnatal period, when normal development of the thalamocortical system is activity dependent, suggests that it may play an important role in synapse formation or stabilization (Katz and Shatz 1996; Shatz 1996). The extremely long duration of the GABA_B IPSP makes it particularly suitable for filtering noncoincident peripheral (or cortical) excitatory inputs. In older rodents, blockade of thalamic GABA_B receptors leads to decreased EEG synchronization (Juhasz et al. 1994), indicating the importance of these receptors in promoting coherent firing of large ensembles of thalamocortical relay neurons. The presence of GABA_A receptors at an early age is a step in the maturation of relay cell properties that eventually permit the burst firing on which thalamic oscillations depend (Warren and Jones 1997).

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Address for reprint requests: R. A. Warren, Centre de recherche Fernand-Seguin, 7331 rue Hochelaga, Montreal, Quebec H1N 3V2, Canada.

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