Synaptic and Synaptically Activated Intrinsic Conductances Underlie Inhibitory Potentials in Cat Lateral Amygdaloid Projection Neurons
In Vivo

E. J. LANG AND D. PARÉ
Département de Physiologie, Université Laval, Québec, Québec, G1K 7P4, Canada

Lang, E. J. and D. Paré. Synaptic and synaptically activated intrinsic conductances underlie inhibitory potentials in cat lateral amygdaloid projection neurons in vivo. J. Neurophysiol. 77: 353–363, 1997. The companion paper demonstrated that the responses of lateral amygdaloid (LAT) projection neurons to the stimulation of major input and output structures are dominated by monophasic hyperpolarizing potentials of large amplitude. To characterize the mechanisms underlying these inhibitory potentials, intracellular recordings of cortically evoked responses were obtained from morphologically and/or physiologically identified LAT projection neurons in barbiturate anesthetized cats. The reversal potential of the cortically evoked hyperpolarization was measured at its peak, and 115 ms later (tail), an interval corresponding to the peak latency of the γ-aminobutyric acid-B (GABA_B) response previously recorded in vitro. When recorded with K-acetate (KAc) pipettes, these reversal potentials were −86.9 ± 1.6 mV (peak; mean ± SE) and −90.7 ± 1.7 mV (tail), suggesting that both Cl− and K+ conductances contribute throughout the cortically evoked hyperpolarization. The small, but consistent, difference between the two reversal potentials suggested that an additional slowly activating K+-mediated component contributed to the inhibitory postsynaptic potential (IPSP) tail. To determine whether Cl− conductances contributed to the evoked hyperpolarization, recordings were performed with KCl; the peak (−57.8 ± 2.2 mV) and tail (−61.3 ± 2.1 mV) reversal potentials were −15–20 mV more depolarized than those recorded with KAc pipettes. However, the difference between the peak and tail reversals remained. In an attempt to block the Cl− conductance, recordings were obtained with pipettes filled with KAc or KCl and 4,4′-disothiocyanostilbene-2,2′-disulfonic acid (DIDS), a Cl− pump blocker that also was reported to block GABA_A responses. With KAc and DIDS, the initial depolarization was prolonged and the amplitude of the hyperpolarization decreased relative to that seen with KAc alone. However, with KCl and DIDS, the reversal potential was shifted to an even greater extent than with KCl pipettes with the evoked response consisting entirely of a large depolarization, which produced a spike burst. These results suggest that LAT neurons have a Cl− pump that is blocked by DIDS, but that their Cl− channels are not blocked by DIDS. To assess the contribution of K+ conductances to cortically evoked hyperpolarizing potentials, recordings were obtained with Cs-acetate pipettes. Under these conditions, the response reversed at more depolarized potentials (peak, −71.9 ± 1.0 mV; tail, −72.0 ± 0.9 mV) compared with KAc recordings, with no difference between the peak and tail reversal potentials. These cells also had depolarized resting potentials (−66.2 ± 1.8 mV) compared with those of cells recorded with KAc pipettes (−73.6 ± 1.8 mV); however, this difference was too small to warrant a redistribution in a redistribution of Cl− ions across the membrane. The action potentials generated by LAT neurons under Cs+ had a shoulder that prolonged their falling phase. The increased duration of the spikes was presumably due to a dendritic Ca2+-conductance because LAT amygdaloid neurons are known to possess such conductances and Cs+ blocks the delayed rectifier and some Ca2+-dependent K+ currents. The dramatic reduction of this shoulder by spontaneous and evoked IPSPs suggests that the activation of dendritic conductances by back-propagating somatic action potentials is regulated tightly by synaptic events. Intracellular injection of the Ca2+ chelating agent, 1,2-bis-(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid (100 mM) caused a depolarization of the peak (−75.3 ± 1.3 mV) and tail (−77.7 ± 1.7 mV) reversal potentials during a time course of 15–45 min. Concurrently, the amplitude of the excitatory postsynaptic potential increased whereas that of the hyperpolarization decreased, suggesting that a Ca2+-dependent K+ conductance contributes significantly to the evoked hyperpolarization. In conclusion, the large hyperpolarizing potentials that regulate the excitability of LAT projection neurons appear to be mediated primarily by a Cl−, presumably GABA_A, IPSP, and a synaptically activated Ca2+-dependent K+ conductance. A relatively weak K+-mediated, possibly GABA_B, IPSP makes a small contribution to the later portions of the response.

INTRODUCTION

The responses of lateral amygdaloid (LAT) neurons are regulated tightly by inhibitory processes, which largely limit orthodromic spiking (Lang and Paré 1997) and may explain their virtual lack of spontaneous activity in unanesthetized cats (Gaudreau and Paré 1996). This inhibition is necessary to counterbalance strong excitatory inputs, which are revealed only when selectively activated with low intensity stimulation of LAT afferents (Lang and Paré 1997). Further, these inhibitory processes appear to be an invariable component of the response of LAT neurons, as they are activated by stimulation of the major input and output structures of the LAT nucleus (Lang and Paré 1997).

In vitro studies have shed some light on the mechanisms underlying these inhibitory processes. LAT neurons were demonstrated to have both γ-aminobutyric acid-A (GABA_A)- and GABA_B-mediated inhibitory postsynaptic potentials (IPSPs) (Sugita et al. 1992, 1993), as well as glycinergic IPSPs (Danover and Pape 1995). The GABAergic IPSPs result primarily from the action of local interneurons (Le Gal La Salle et al. 1978), whereas the source of the glycinergic innervation has yet to be identified. The GABA_A- and GABA_B-mediated IPSPs were shown to have distinct time courses and reversal potentials (Sugita et al. 1992, 1993) that were similar to those found in other amygdaloid nuclei (Nose et al. 1991; Rainnie et al. 1991; Washburn and Moises 1992) and elsewhere in the nervous system.
(Dutar and Nicoll 1988; Hirsch and Burnod 1987; McCormick 1989; Soltesz et al. 1988). Thus the GABA_A IPSPs were shown to be mediated by a Cl⁻ conductance, reversed around −70 mV, had rapid times to peak (10–35 ms), and had short durations (50 ms), whereas GABA_B IPSPs were found to be generated by a K⁺ conductance, reversed around −110 mV, had long times to peak (120–170 ms), and had long durations (350–1,500 ms) (Sugita et al. 1993).

The differences in the shape and reversal potentials of the evoked responses observed in vivo (Lang and Paré 1997) from those reported in vitro led us to investigate the conductances underlying the cortically evoked hyperpolarizing responses of LAT neurons. Our results suggest that the major inhibitory processes controlling LAT neuronal activity in vivo are a Cl⁻-mediated, presumably GABA_A, IPSP and a Ca²⁺-dependent K⁺ [K_Ca] conductance, the latter being activated primarily by synaptic input, rather than by action potentials. GABA_A IPSPs appear to make only a small contribution to the response.

METHODS

Intracellular recordings were obtained from cats (2.5–3.5 kg) anesthetized with sodium pentobarbital (Somnotol, 40 mg/kg ip). The surgical, recording, stimulation, analysis, and histological procedures are described in the companion paper (Lang and Paré 1997).

The recording electrodes consisted of glass capillary tubes pulled to a tip diameter of 0.5 µm (35–45 MΩ). Pipettes were filled with various electrolytic solutions: K-acetate (KAc; 4 M), Cs-acetate (CsAc; 3 M), or KCl (3 M). In some experiments, one of the following drugs were added to the electrolytic solution: N-(2,6-dimethyl-phenylcarbamoylmethyl)-triethylammonium bromide (QX-314; 50 mM) to block Na channels (Connors and Prince 1982; Strichartz 1973) and increase the input resistance (R_in); 1,2-bis(2-aminophenoxo)ethane-N,N,N ′,N ′'-tetraacetic acid (BAPTA; 100 mM) to investigate the contribution of Ca²⁺-dependent conductances; and 4,4'-disothiocyanato-2,2'-stilbenedisulphonic acid (DIDS; 200 µM) to block the Cl⁻ transporter. In some experiments, Neurobiotin (14 mg/ml; Vector Labs) was added for intracellular labeling of recorded neurons. The methods used to calculate the IPSP reversal potentials and estimate the R_in before and during the IPSPs are described in the companion paper.

RESULTS

Stable intracellular recordings were obtained from 174 LAT neurons with physiological properties known to be characteristic of LAT projection cells (Paré et al. 1995a). In agreement with this, 22% of them were formally identified as projection cells on the basis of the morphological and/or physiological criteria described in the companion paper (Lang and Paré 1997). LAT neurons with fast-spiking or bursting firing patterns were not included in the present study. All cells had spike amplitudes between 60 and 90 mV and, with the exception of KCl recordings, stable resting potentials (V_R) of −60 mV or greater. Cells typically were held for 0.5–2 h.

Reversal potentials of evoked hyperpolarizations suggest combined action of K⁺ and Cl⁻ conductances

Entorhinal (ENT-) or perirhinal (PRH-) evoked responses were analyzed in 74 neurons recorded intracellularly with KAc pipettes. As described in the preceding paper (Lang and Paré 1997), cortically evoked responses consisted of a short-latency excitatory postsynaptic potential (EPSP) followed by a monophasic hyperpolarization of large amplitude (±20 mV from rest) and long duration (±1.5 s). Consistent with the monophasic aspect of the evoked hyperpolarization, analysis of R_in changes revealed that after dropping to a minimum at the IPSP peak, the R_in increased monotonically back to baseline (not shown). R_in drops increased with stimulation intensity and could reach up to 85% of the resting R_in.

A detailed quantitative analysis of cortically evoked IPSPs was performed in a representative sample of these neurons (n = 11). These cells had an average V_R of −73.5 ± 1.8 mV (n = 11; mean ± SE). The average peak reversal potential was −86.9 ± 1.6 mV (n = 11), a value intermediate between the reversal potentials typically found for Cl⁻- and K⁺-mediated IPSPs, suggesting that the hyperpolarization was generated by a combination of Cl⁻ and K⁺ conductances.

To determine whether the relative contributions of these conductances changed during the course of the response, the reversal potential was measured at the peak and 115 ms later, the interval between the peak of the GABA_A- and GABA_B-mediated IPSPs reported for amygdaloid neurons in vitro (Washburn and Moises 1992). Comparison of reversal potentials at the peak and tail revealed small, but consistent differences (Table 1). In every case, the tail reversal was more negative than the peak one, with an average difference of 3.8 mV (P < 0.001, paired t-test, n = 11).

KCl produces a positive shift of the peak and tail reversal potentials

The contributions of Cl⁻ conductances to cortically evoked responses were first investigated with KCl pipettes in 21 neurons. A detailed quantitative analysis of cortically evoked IPSPs was performed in a representative sample of these neurons (n = 5). These cells had more depolarized V_R (−58.8 ± 1.4 mV, n = 5) and lower R_in (13.1 ± 1 MΩ, n = 5) than cells recorded with KAc (see Table 1). At depolarized levels, PRH stimulation typically evoked a short latency EPSP that was truncated by a shallow monophasic hyperpolarization of long duration. The hyperpolarization reversed near the V_R (−57.8 ± 2.2 mV; tail, −61.3 ± 2.1 mV; n = 5). However, despite the large positive shift from KAc values, a similar difference between the peak and tail reversal potentials, 3.5 ± 2.1 mV, remained. Examples of cortically evoked responses recorded with a KCl pipette are shown in Fig. 1A1. In this case, the response had reversals of −62.6 mV (peak, ◇) and −65.1 mV (tail, ▲; Fig. 1A2) and produced a 74% decrease in R_in at its peak (Fig. 1A3).

To rule out the possibility that the low R_in of cells recorded with KCl pipettes (Table 1) prevented observation of electrotonically remote events, recordings were obtained with pipettes containing KCl and QX-314. Under these conditions, cells had significantly higher R_in (31.1 ± 5.8 MΩ; n = 4, P < 0.05). Nevertheless, the evoked IPSPs were monophasic and had reversal potentials that were similar to those obtained with KCl pipettes (peak, −58.5 ± 1.9 mV;
BAPTA/K-Ac 9
Cs-Ac 9
KCl 5

DIDS (was decreased (Fig. 2C). B3)
N-phenylcarbamoylmethyl)-triethylammonium bromide; Cs-Ac, Cs acetate; BAPTA, 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid.

Pipette solution is indicated in left-most column (Condition). Reversal potentials were measured at the peak of the hyperpolarization (Peak) and 115 ms later (Tail). ΔREV is the average difference between the two reversal potentials (Tail-Peak). K-Ac, K-acetate; QX-314, N-(2,6-dimethylphenylcarbamoylmethyl)-triethylammonium bromide; Cs-Ac, Cs acetate; BAPTA, 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid.

Table 1. Summary of resting potentials, input resistances, and reversal potentials

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>$V_R$, mV</th>
<th>$R_{IN}$, MΩ</th>
<th>Peak, mV (○)</th>
<th>Reversal Potential</th>
<th>ΔREV, mV (▲ - ○)</th>
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<tr>
<td>K-Ac</td>
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<td>22.32</td>
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<td>-3.52</td>
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<td></td>
<td></td>
<td>(1.36)</td>
<td>(0.97)</td>
<td>(2.23)</td>
<td>(2.14)</td>
<td>(0.09)</td>
</tr>
<tr>
<td>KCl/QX-314</td>
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<td>-58.49</td>
<td>-61.89</td>
<td>-3.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.08)</td>
<td>(5.77)</td>
<td>(1.94)</td>
<td>(2.97)</td>
<td>(1.03)</td>
</tr>
<tr>
<td>Cs-Ac</td>
<td>9</td>
<td>-66.20</td>
<td>23.66</td>
<td>-71.94</td>
<td>-72.01</td>
<td>-0.07</td>
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<tr>
<td></td>
<td></td>
<td>(1.75)</td>
<td>(1.22)</td>
<td>(1.03)</td>
<td>(0.91)</td>
<td>(0.52)</td>
</tr>
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<td>BAPTA/K-Ac</td>
<td>9</td>
<td>-68.13</td>
<td>25.46</td>
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<td>-77.71</td>
<td>-1.98</td>
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<td></td>
<td></td>
<td>(2.48)</td>
<td>(1.58)</td>
<td>(1.04)</td>
<td>(1.51)</td>
<td>(0.80)</td>
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</table>

Cl⁻-mediated IPSPs are not blocked by DIDS in LAT neurons

Intracellular injection of DIDS was used in an attempt to block the Cl⁻ conductance as DIDS has been reported to block GABA_A responses in some neurons (Nelson et al. 1994; Sun and Reis 1995). In LAT neurons recorded with pipettes containing KAc and DIDS (n = 5), the duration and amplitude of the initial depolarization were increased whereas the amplitude of the subsequent hyperpolarization was decreased (Fig. 2C) relative to that seen with KAc pipettes (Fig. 2D). However, the reversal potentials (peak, -79.7 ± 0.3 mV; tail, -82.8 ± 0.5 mV; n = 5; P < 0.01; paired t-test) shifted positively, thus raising the possibility that DIDS was acting by blocking a Cl⁻ pump. Therefore recordings were made with pipettes containing KCl and DIDS (n = 5), where if DIDS was acting by blocking Cl⁻ channels, the shift in reversal potential induced by KCl should have been abolished. In contrast with this, DIDS potentiated the positive reversal potential shift induced by KCl (compare Fig. 2, A and B), indicating that DIDS does not block Cl⁻ channels but rather a Cl⁻ pump and that Cl⁻ conductances contribute throughout the IPSP. It was not possible to measure the reversal potential of responses recorded with pipettes containing KCl and DIDS because the large depolarizing responses triggered bursts of spikes that often were characterized by a failure of the somatodendritic component (Fig. 2A). These spike bursts were seen only with pipettes containing KCl and DIDS; under all other recording conditions, cortical stimuli elicited at most a single spike.

Elimination of peak-tail shift in reversal potential with CsAc

To investigate the contribution of K⁺ conductances to the PRH-evoked hyperpolarization, intracellular recordings were obtained with CsAc pipettes (n = 38), as intracellular Cs⁺ has been reported to block GABA_A IPSPs (Gähwiler and Brown 1985; Otis et al. 1993). These cells had more depolarized $V_R$ (-66.2 ± 1.8 mV; n = 9) than neurons recorded with KAc pipettes (P < 0.01) but no significant difference in $R_{IN}$ (Table 1). Although the shape and time course of these evoked IPSPs were similar to those recorded with KAc pipettes, they had more depolarized reversal potentials (peak, -71.9 ± 1.0 mV; tail, -72.0 ± 0.9 mV; n = 9) and there was no difference between the peak and tail reversal potentials (-0.1 ± 0.5 mV). Responses to PRH stimulation recorded with a CsAc pipette are shown in Fig. 3A. In this case, the inhibitory response had a peak reversal potential (○) of -75.9 mV and a tail reversal (▲) of -76.6 mV (Fig. 3C).

The effect of Cs⁺ on the peak (Fig. 4A) and tail (Fig. 4B) reversal potentials is summarized in Fig. 4, where the response amplitude (△V) is plotted against $V_R$ for cells recorded with either KAc (n = 11, ○) or CsAc (n = 9, ○) electrodes. Linear fits to the data gave reversal potentials of -86.2 mV (peak) and -91.3 mV (tail) with KAc as compared with -72.1 mV (peak) and -72.9 mV (tail) with CsAc pipettes.

Typically, the effects of Cs⁺ developed gradually during 5–15 min and were related to the amount of positive current passed through the electrode. For example, the reversal potentials shown in Fig. 3 were obtained ≈60 min after penetration of the cell. Responses evoked immediately after stabilization of the cell (3 min) had reversal potentials of -92.5 mV (peak) and -97.1 mV (tail). Thus before Cs⁺ had a chance to diffuse throughout the cell, both the peak and tail reversal potentials as well as the difference between them (-4.7 mV) were similar to those obtained with KAc pipettes.
FIG. 1. Perirhinal (PRH)-evoked responses recorded with pipettes containing KCl (A) or KCl and QX-314 (B). A1: synaptic responses studied at different membrane potentials \( V_{m} \) determined by DC current injection. At \(-56 \) mV, inhibitory postsynaptic potential (IPSP) peak occurred 63 ms after onset (●) and 115 ms later (tail; ▲). A2: plot of IPSP amplitude (\( \Delta V \)) vs. \( V_{m} \) at IPSP peak (●). A3: plot of voltage change from \( V_{R} \) (\( \Delta V \)) vs. DC current (I) before PRH stimuli (●), at IPSP peak (○) and 115 ms later (tail; ▲). Slopes of fitted lines give \( R_{in} \) at each time. Unless otherwise stated, all traces in this and succeeding figures are average of 5–10 responses. B: PRH-evoked responses recorded with pipettes containing KCl and \( N\)-(2,6-dimethyl-phenylcarbamoylmethyl)-triethylammonium bromide (QX-314). B1: synaptic responses evoked from different \( V_{m} \) ranging from \(-48 \) to \(-94 \) mV induced by DC current injection. Note absence of action potentials at more depolarized levels is due to action of QX-314. B2: plot of IPSP amplitude (\( \Delta V \)) vs. \( V_{m} \) at IPSP peak (●) and tail (▲). B3: plot of voltage change from \( V_{R} \) (\( \Delta V \)) vs. current (I) before PRH stimuli (●), at IPSP peak (○), and tail (▲). Note significantly higher \( R_{in} \) at rest as compared with KCl recording in previous figure.

The \( I-V \) curves at the peak amplitude of the hyperpolarization for the four recording conditions (KAc, CsAc, KCl, KCl + QX-314) are compared in Fig. 5. The linear fits for the KCl and KCl + QX-314 conditions were very similar and gave nearly identical reversal potentials of \(-58.0 \) and \(-57.6 \) mV, respectively. In contrast, the linear fits in the Cs-Ac and K-Ac conditions yielded more negative reversal potentials of \(-72.1 \) and \(-86.2 \) mV. The least-square fits to the data yielded highly significant correlation coefficients \(( P < 0.01 )\) of 0.90 (K-Ac), 0.93 (Cs-Ac), 0.91 (KCl), and 0.81 (KCl + QX-314).

\( Cs^{+} \) increases action potential duration in lateral amygdaloid neurons

In addition to shifting the reversal potentials of the IPSPs, \( Cs^{+} \) altered the shape of the action potential. Whereas the half-amplitude duration of the spike was 1.38 ms on average in neurons recorded with KAc pipettes (Paré et al. 1995), in cells recorded with CsAc pipettes, the spike duration increased by an order of magnitude or more. This increase was partially reversible and was time and voltage dependent. Thus after a period of hyperpolarization, the spike duration

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The image contains a figure with labeled parts A1, A2, A3, B1, B2, B3, each containing graphs with plots of membrane potential (V) versus current (I), and voltage change (ΔV) versus membrane potential (V_m) or current (I). The graphs illustrate synaptic responses at different membrane potentials and voltage changes induced by DC current injection. The figures are labeled with annotations and measurement details, including reversal potentials, slopes, and average responses. The text accompanying the figure discusses the effects of KCl and KCl + QX-314 on synaptic responses and action potentials in lateral amygdaloid neurons.
appearance of a broad shoulder, which interrupted the spike repolarization (Fig. 3B).

To investigate the effects of PRH-evoked IPSPs on the spike shape, cells were depolarized steadily to a level sufficient to elicit action potentials in the absence of detectable synaptic events (Fig. 6). We compared these action potentials to those elicited by PRH stimulation and those occurring spontaneously in relation to ENT sharp potentials (SPs); (Fig. 6A, curved arrow). This is shown in Fig. 6A where PRH-evoked (Fig. 6, A1 and B1), current induced (Fig. 6, A2 and B2) and SP-related (Fig. 6, A3 and B3) spikes are seen. The current-induced action potentials had the longest duration (9.1 ± 0.1 ms; n = 13) whereas the SP-related (5.4 ± 0.4 ms; n = 4) and PRH-evoked (4.0 ± 0.2 ms; n = 8) spikes were significantly shorter in duration (P < 0.001; Fig. 6B4).

**Time-dependent reduction of cortically evoked hyperpolarization by BAPTA**

The results of the CsAc recordings suggested that K⁺ currents make an important contribution throughout the hyperpolarization. However, the peak of the response occurred too rapidly to be mediated by GABAB receptors. Therefore we investigated the possibility that a K(Ca) conductance contributed to the evoked response by recording with pipettes was reduced toward KAc values, whereas subsequent maintained depolarization led to a return to the increased duration. This increase in spike duration was associated with the appearance of a broad shoulder, which interrupted the spike repolarization (Fig. 3B).

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containing KAc and BAPTA (50 mM; n = 23). The effect of BAPTA on cortically evoked hyperpolarizations developed gradually during 15–45 min. During this period, the amplitude of the EPSP increased (31.5 ± 1.6%, n = 3, P < 0.05), that of the hyperpolarization decreased by (33 ± 11%, n = 3, P < 0.01), and the reversal potential shifted to more positive values (peak, −75.7 ± 1.0 mV; tail, −77.7 ± 1.5 mV, P < 0.01). In parallel with this, the R\text{IN} drop related to the IPSP diminished (28 ± 3.1%, n = 3, P < 0.05). However, the difference between the reversal potentials at the peak (−75.7 ± 1.0 mV) and tail (−77.7 ± 1.5 mV) remained (P < 0.01, paired t-test, n = 9). The time-dependent effect of BAPTA on PRH-evoked responses is illustrated in Fig. 7. The hyperpolarization decreased in amplitude by 49% during 44 min (Fig. 7, A and B1) whereas the EPSP grew in amplitude ≈33% of control (Fig. 7, A, inset, and B2). In this case, the peak reversal potential was initially −86.9 mV, typical for KAc pipettes but fell to a value of −76.1 mV after 44 min (Fig. 7B1). In parallel with this, the amplitude of afterhyperpolarizing potential (AHP) that followed current-evoked spikes gradually diminished (Fig. 7A, bottom inset) and the R\text{IN} drop related to the IPSP decreased by 32.6% (not shown).

Because the effects of BAPTA suggested that LAT neurons are endowed with a large K(Ca) conductance that can be activated in the absence of somatic spikes (Fig. 7), we investigated whether orthodromic spikes could further increase the activation of this conductance. LAT neurons recorded with KAc pipettes exhibit AHPs following single spikes (Fig. 8, A1 and A3) and depolarizing current pulses (Fig. 8A1). In the latter case, the AHP amplitude was related to the size of the current-induced depolarization (Fig. 8A2). The intensity of the PRH stimuli was set to elicit spikes with a probability of ≈50%. The average PRH-evoked responses with and without an evoked spike are superimposed in Fig. 8B, with a slow (Fig. 8B1), intermediate (Fig. 8B2), and fast (Fig. 7B3) time base. As shown in Fig. 8B, the sub- and suprathreshold responses to PRH stimuli are virtually identical. Only during the initial 5–10 ms was there an additional hyperpolarization due to the action potential AHP (Fig. 8B3).

**Discussion**

Synaptically evoked inhibitory potentials seem to play a major role in determining the firing behavior of LAT projection neurons (Gaudreau and Pare 1996). The significant phenomenological differences existing between these potentials under in vivo (Lang and Paré 1997) and in vitro (Sugita et al. 1992, 1993) conditions led us to investigate the mechanisms underlying cortically evoked inhibition using an in vivo intracellular preparation. Our results suggest that the cortically evoked hyperpolarizing responses are composed primarily of two components: a Cl−, presumably GABA\text{A}-mediated, IPSP and a synaptically activated intrinsic conductance, possibly a K(Ca) conductance. A small, K+–mediated component that occurred at a long latency, and may correspond to a GABA\text{A} IPSP, also contributed to the hyperpolarization.

**Responses to PRH stimulation involve Cl−-mediated IPSPs**

The hyperpolarizing responses of LAT neurons to cortical stimuli reversed around −85 mV when recorded with KAc pipettes. This value, intermediate between those typically found for Cl−- and K+–mediated IPSPs in various types of neurons (Dutar and Nicoll 1988; Hirsch and Burnod 1987; Rainnie et al. 1991; Sugita et al. 1992, 1993; Washburn and Moises 1992), suggests the combined contribution of Cl− and K+ conductances to the cortically evoked hyperpolarizations. Evidence of a significant Cl− component was obtained with KCl pipettes, as they produced a large (≥25 mV) positive shift of the reversal potential. The shift of both the peak and tail reversals (and even ones measured later in the response—data not shown) indicates that this conductance contributes throughout the hyperpolarization.

The Cl− conductance described here probably corresponds to the GABA\text{A} IPSP described in neurons of the LAT nucleus maintained in vitro (Sugita et al. 1992, 1993). However, a Ca2+-dependent Cl− conductance also has been demonstrated in these neurons (Sugita et al. 1993) and may...
have contributed as well. However, this latter conductance probably comprises a relatively small percentage of the total Cl⁻ conductance, because a significant hyperpolarization was evoked in cells filled with BAPTA, where Ca²⁺-dependent conductances should have been eliminated. More importantly, the reduction in the amplitude of the evoked hyperpolarization induced by BAPTA was associated with a positive shift in reversal potential, whereas the block of a Cl⁻ conductance should have produced the opposite effect.

Two factors may account for the longer duration of the Cl⁻-mediated IPSP in vivo. First, the widespread divergence of activity through cortical and amygdaloid networks probably resulted in a temporally distributed activation of inhibitory interneurons of the LAT nucleus (Lang and Pare 1997). Second, barbiturates are known to increase the duration of GABA\(_A\) IPSPs (Thompson and Gähwiler 1992).

\[ A \text{ Cl}^- \text{ pump maintains a low intracellular Cl}^- \text{ concentration in LAT neurons} \]

To further assess the contribution of Cl⁻ currents to the hyperpolarization, we used pipettes containing DIDS, an
inhibitor of the Cl\textsuperscript{−} transporter (Cabantchik and Rothstein 1974; Russell and Brodwick 1979) that has been reported to block \textit{GABA\textsubscript{A}} responses (Nelson et al. 1994; Sun and Reis 1995) as well as voltage-sensitive Cl\textsuperscript{−} channels (Kokubun et al. 1991; Miller and White 1984) in some neurons. However, our results indicate that DIDS does not block Cl\textsuperscript{−} channels in LAT neurons, because it produced positive shifts in the reversal potentials. This is opposite to what would be expected after blockade of Cl\textsuperscript{−} channels given the contribution of Cl\textsuperscript{−} and K\textsuperscript{+} currents to the PRH-evoked hyperpolarization (see below). Rather, the positive shifts of the reversals were consistent with inhibition of a Cl\textsuperscript{−} transporter that normally maintains a low intracellular Cl\textsuperscript{−} concentration.

The fact that DIDS had a much larger effect on the reversal of cortically evoked hyperpolarizations when used with KCl than KAc suggests that the Cl\textsuperscript{−} pump of LAT neurons has a high transport capacity. In light of recent findings suggesting that inhibitory interneurons contribute a tonic GABAergic input to projection cells (Pare and Gaudreau 1996), this high transport capacity may be necessary to maintain a Cl\textsuperscript{−} gradient that allows \textit{GABA\textsubscript{A}} receptors to mediate sustained hyperpolarizing responses.

\textit{GABA\textsubscript{B}} responses of LAT neurons are relatively minor in vivo

Despite the monophasic nature of the hyperpolarization recorded in vivo, in vitro results (Sugita et al. 1992, 1993) suggested that there should be both an early \textit{GABA\textsubscript{A}} and a late \textit{GABA\textsubscript{B}} component. Therefore, reversal potentials were calculated at the peak of the response, whose latency (≈50 ms) was too short to be significantly contaminated by a \textit{GABA\textsubscript{A}} IPSP, and 115 ms later (tail), an interval corresponding to the time separating the peaks of the \textit{GABA\textsubscript{A}} and \textit{GABA\textsubscript{B}} responses recorded in vitro (Washburn and Moises 1992). The small (≈3.5 mV) but consistent difference found between these two reversal potentials could have resulted from an increase in the contribution of K\textsuperscript{+} currents, a decreased involvement of Cl\textsuperscript{−} currents, or from the tapering of the EPSP during this interval. However, the elimination of the difference in the reversal potentials by Cs\textsuperscript{+}, which blocks various K\textsuperscript{+} channels including the one mediating the \textit{GABA\textsubscript{A}} IPSP (Gäwiler and Brown 1985), indicates that it was primarily due to a slowly activating K\textsuperscript{+} conductance, possibly a \textit{GABA\textsubscript{B}} response.

In basolateral amygdaloid neurons, \textit{GABA\textsubscript{B}} synapses have been shown to occur preferentially along the distal dendrites,
whereas GABA_{B} inputs dominate somatically (Washburn and Moises 1992). Assuming that a similar distribution exists in LAT neurons, it is possible that the low R_{IN}S of cells recorded in vivo combined with the perisomatic action of GABA (Carlsen 1988) limited the observation of the GABA_{B} IPSPs. However, in cells with higher R_{IN}S, recorded with pipettes containing QX-314, the hyperpolarization remained monophasic with the difference between the peak and tail reversal potentials virtually unchanged. Because QX-314, blocks some K^{+} conductances (Andreasen and Hablitz 1993; Perkins and Wong 1995) and, in particular, has been reported to block GABA_{B} IPSPs in hippocampal neurons (Nathan et al. 1990), the failure to observe a larger GABA_{B} response in these cells may have been due to a partial block of GABA_{B}-activated K^{+} channels. However, even this seems unlikely, as an effect equal and opposite to that produced by the increase R_{IN} would be required, because the difference between the peak and tail reversal potentials was not abolished by QX-314. Thus our results suggest that there might be a relatively weak GABA_{B} response in vivo, which appears to contribute to the latter part of the cortically evoked hyperpolarization.

**A synaptically activated intrinsic conductance controls the synaptic responsiveness of LAT neurons**

Recording cortically evoked responses with CsAc pipettes induced a large positive shift (≈15 mV) of the peak and tail reversal potentials relative to KAc recordings. This shift was too large to be attributed only to the redistribution of Cl^{-} ions across the membrane as a result of the change in V_{k}, particularly given the evidence of an effective Cl^{-} pump, which would oppose such redistributions. Rather, the effects of Cs^{+} suggest the presence of a significant K^{+}-mediated component to the cortically evoked hyperpolarization at a latency too short to be ascribed only to a GABA_{B} IPSP. Because Cs^{+} is known to block K_{(Ca)} channels (Yellen 1987) and because these channels underlie phenomenologically similar events (e.g., AHPs) (for review, Sah 1996), we investigated whether K_{(Ca)} conductances could be involved in the evoked hyperpolarization.

Intracellular injection of BAPTA decreased the amplitude of the hyperpolarization, induced a positive shift of its reversal potential, and decreased the R_{IN} drop related to the IPSP, thus suggesting the contribution of a Ca^{2+}-dependent process to the cortically evoked response. The amount of Cl^{-} conductance activated by GABA in hippocampal neurons is known to be controlled by a competition between a Ca^{2+}-dependent dephosphorylation that reduces the conductance and a phosphorylation process that maintains it (Chen and Wong 1994, 1995; Stelzer et al. 1988). In our experiments, if BAPTA had acted by reducing the Ca^{2+}-dependent dephosphorylation, the R_{IN} drop related to the IPSP should have increased. In contrast, the R_{IN} drop decreased and AHP amplitudes diminished, thus suggesting that BAPTA acted by inhibiting a K_{(Ca)} current. Interestingly, orthodromic spikes did not increase the size of the hyperpolarization, suggesting that cortical synapses have a preferential access to this conductance.

In agreement with the above, similar findings were obtained recently in LAT neurons recorded in vitro (Danobe and Pape 1996). Furthermore, in this study, application of drugs known to modulate K_{(Ca)} currents similarly modulated the synaptically evoked slow hyperpolarization.

The similarity between the Cs^{+} and BAPTA-induced shifts in reversal potentials from KAc values suggests that a K_{(Ca)} current is the main K^{+} current contributing to PRH-evoked hyperpolarizations. However, Cs^{+}- or BAPTA-resistant K^{+} currents also may have contributed to the response. The small difference between the Cs^{+} and the BAPTA reversals could reflect one or more of the following factors: the presence of a weak GABA_{B} IPSP unaffected by BAPTA, Ca^{2+}-independent K^{+} channels blocked by Cs^{+}, and blockage of a Ca^{2+}-dependent Cl^{-} conductance by BAPTA (Sugita et al. 1993).

The alteration of the EPSP rising phase by the intracellular diffusion of BAPTA indicates that this K_{(Ca)} current is activated within a few milliseconds of the response onset and suggests that the K_{(Ca)} channels and those permitting Ca^{2+} entry are in close proximity. A similar conclusion has been
reached for N-type Ca$^{2+}$ and SK$_{(Ca)}$ [small conductance K$_{(Ca)}$] channels in hypoglossal motor neurons (Viana et al. 1993). Such an arrangement is necessitated by the limited diffusion that can occur in such a short interval (Sah 1996) and ensures that the K$_{(Ca)}$ channels are within the spatially limited microdomains of high concentration that form after Ca$^{2+}$ entry (Llinás et al. 1992).

Several possibilities exist for the source of the Ca$^{2+}$ that activates the K$_{(Ca)}$ channels. The slow development of the effect of BPATa suggests a dendritic location for at least a significant fraction of these channels. In agreement with this, in vitro studies of amygdaloid cells have demonstrated the presence of high-threshold Ca$^{2+}$ conductances located in the dendrites (Foehring and Scroggs 1994). These conductances could be activated by cortically evoked EPSPs. In addition, the EPSPs themselves may be the source of Ca$^{2+}$ entry, particularly if they involve N-methyl-D-aspartate channels, which are known to be permeable to Ca$^{2+}$ (Ascher and Nowak 1986). Consistent with the latter possibility, the cortically evoked hyperpolarizing potential is markedly diminished under ketamine/xylazine anesthesia (unpublished observations). In either case, the temporally dispersed arrival of cortical afferent activity to the LAT nucleus, may contribute to the prolonged activation of the K$_{(Ca)}$ current.

**Synaptic inputs limit the activation of dendritic conductances by action potentials**

The action potentials generated by LAT neurons under Cs$^{+}$ are similar to those of inferior olivary cells in having a shoulder that prolongs the falling phase of the spike (Crill 1970; Llinás and Yarom 1981a). In inferior olivary cells, this shoulder is mediated by the activation of a high-threshold Ca$^{2+}$ conductance located in their dendrites (Llinás and Yarom 1981a,b). Because LAT neurons have been shown to possess such dendritic conductances, it is possible that a similar mechanism underlies the shoulder observed here under Cs$^{+}$ as this ion blocks the delayed rectifier and some Ca$^{2+}$-dependent K$^+$ currents (Hille 1992; Yellen 1987). By blocking these K$^+$ currents, Cs$^+$ reduced the resistance drop usually occurring during the action potential repolarization, thus maximizing the dendritic depolarization provoked by the somatic spike and allowing the cell body to “see” more of the active dendritic events triggered by the back-propagating spike. This mechanism coupled with the relatively slow inactivation of high-threshold Ca$^{2+}$ conductances (Bean 1989; Kay 1991) probably explain the long duration of action potentials under Cs$^{+}$.

The dramatic reduction of this shoulder by cortical stimulation suggests that the activation of dendritic conductances by back-propagating somatic action potentials (Stuart and Sakmann 1994) is regulated tightly by synaptic events.

**Synaptic and synaptically activated intrinsic conductances regulate the excitability of LAT projection neurons**

The results of the present study indicate that the excitability of LAT projection cells is regulated tightly by a combination of synaptic and synaptically activated intrinsic conductances. This conclusion fits well with recent data indicating that these neurons are virtually silent in unanesthetized animals (Gaudreau and Paré 1996). The question then arises as to why it is so critical to dampen the excitability of this brain region that multiple mechanisms are employed to this end. The answer may reside in the powerful excitatory projections that this nucleus provides to widespread cortical structures, which if not tightly controlled can lead to the generation of epileptic events.

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