Cell-Type-Specific GABA Responses and Chloride Homeostasis in the Cortex and Amygdala

MARZIA MARTINA, SÉBASTIEN ROYER, AND DENIS PARÉ
Laboratoire de Neurophysiologie, Département de Physiologie, Faculté de Médecine, Université Laval, Québec, Quebec G1K 7P4, Canada

Received 13 April 2001; accepted in final form 20 August 2001

INTRODUCTION

Within neural networks, excitation and inhibition must be tightly regulated to allow adaptive computations while preventing epileptic activity. To maintain this narrow operactive range, the brain has evolved with a large variety of inhibitory neurons. In the cerebral cortex for instance, several types of GABAergic interneurons have been identified, each with its particular complement of voltage-dependent conductances, molecular markers, and afferent connectivity (Cauli et al. 1997; Freund and Buzsáki 1996; Gupta et al. 2000; Kawaguchi and Kubota 1997; Rudy and McBain 2001; Somogyi et al. 1998).

One major class of interneurons, common to the neocortex (Connors et al. 1982; McCormick et al. 1985), perirhinal cortex (Faulkner and Brown 1999; Martina et al. 2001), and basolateral amygdala (Lang and Paré 1998; Paré et al. 1995; Rainnie et al. 1993; Washburn and Moises 1992a), are fast-spiking (FS) cells. In response to depolarization, FS cells can sustain high firing rates with little or no accommodation. In contrast, most pyramidal cells display various degrees of spike frequency accommodation, a firing pattern termed regular spiking (RS).

In the course of experiments on the perirhinal network (Martina et al. 2001), we have obtained evidence suggesting that other factors than intrinsic membrane properties contribute to render FS cells more excitable than RS neurons. Indeed, we have observed that FS cells, in contrast with RS neurons, lacked overt inhibitory responses to afferent activation. The present study was undertaken to assess the generality of this phenomenon and identify the underlying mechanisms by performing whole cell recordings of FS and RS neurons in the basolateral amygdala, widely regarded as a cortex-like structure (Carlsen and Heimer 1988), as well as in the parietal and perirhinal cortices.

Our results suggest that in these three brain regions, FS and RS cells generate contrasting responses to the inhibitory transmitter GABA because they are endowed with different complements of GABA receptors (A vs. A and B) and chloride homeostatic mechanisms.

METHODS

Preparation of amygdala, perirhinal, and neocortical (parietal) slices

Coronal brain slices were obtained from Hartley guinea pigs (250–300 g; 21– to 28-days old). Prior to decapitation, the animals were anesthetized with pentobarbital (40 mg/kg ip) and ketamine (100 mg/kg ip), in agreement with the guidelines of the Canadian council on animal care. The brain was removed and placed in an oxygenated physiological solution [artificial cerebrospinal fluid (ACSF); 4°C] containing (in mM) 126 NaCl, 2.5 KCl, 1.25 NaHPO₄, 1 MgCl₂, 2 CaCl₂, 26 NaHCO₃, and 10 glucose. A block containing the region of interest was prepared, and sections (400 µm) were obtained with a vibrating microtome. The slices were stored for 1 h in an oxygenated chamber at 23°C. One slice was then transferred to a recording chamber perfused with an oxygenated ACSF (2 ml/min). The temperature of the chamber was gradually increased to 32°C before the recordings.

Data recording and analysis

Two recording methods were used: whole cell and perforated patch. Whole cell recordings were obtained with borosilicate pipettes filled with a solution containing (in mM) 130 K-gluconate, 10 N-2-hydroxy-
ethylpiperazine-N'-2-ethanesulfonic acid, 10 KCl, 2 MgCl$_2$, 2 ATP-Mg, and 0.2 GTP-tris(hydroxy-methyl)aminomethane. In some experiments, Neurobiotin (0.5%) was added to the intracellular solution for morphological identification of the cells (see following text). pH was adjusted to 7.2 and osmolality to 280–290 mOsm. With this solution, the liquid junction potential was measured (10 mV), and the membrane potential ($V_m$) was corrected accordingly. The pipettes had resistances of 4–8 MΩ when filled with this solution. Bridge balance was monitored regularly, and recordings with series resistance higher than 15 MΩ were discarded.

The cation-selective ionophore gramicidin was used for perforated-patch recordings to prevent interference with the intracellular chloride concentration (Myers and Haydon 1972; Ulrich and Huguenard 1997). A stock solution of gramicidin (5 mg/ml in DMSO) was prepared, sonicated for 30 s, and added to the prefilled pipette solution (5–10 μg/ml). The composition of the intracellular solution was identical to that described in the preceding text with the proviso that K-glucuronate was replaced with an equimolar amount of KCl. This change was implemented to facilitate detection of spontaneous shifts in recording modes (rupture of the membrane). With this solution, the liquid junction potential was measured (2 mV) and the $V_m$ corrected accordingly. Two precautions were used to minimize gramicidin ejection from the patch pipette when approaching the cells: we used as little positive pressure as possible and the pipette tip was filled with the normal intracellular solution (the gramicidin-containing solution was added by backfilling). Removal of the positive pressure usually led to the formation of a high resistance seal ($>1$ GΩ). Perforated patches ($>75$ MΩ resistances) were obtained after ~30 min. Current-clamp recordings were obtained under visual control using differential interference contrast and infrared video microscopy. To increase the likelihood of obtaining recordings from the comparatively less numerous FS cells, patch pipettes were aimed for small-diameter somatic profiles (~10 μm). The proportion of FS cells climbed significantly using this method.

Electroresponsive properties were investigated by applying 0.2- to 5-s current pulses from rest and one or more prepulse potentials as determined by steady current injection. GABAergic afferents to the recorded neurons were activated by brief (50–300 μA) bipolar electrical stimuli (0.1–1 mA) applied through tungsten electrodes (80 μm diam; 80 kΩ).

Concentrations of drugs applied in the perfusate were (in μM) 10 bicuculline, 100 saclofen, 20 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), 100 picrotoxin, 100 (−)-2-amino-5-phosphonopentanoic acid (AP-5), 20 bumetanide, 1,000 furosemide, and 0.5 tetrodotoxin (TTX). All drugs were obtained from RBI (Natick, MA) with the exceptions of saclofen (Tocris, Bristol, UK). The free base version of bicuculline was used as N-methyl derivatives of this drug block apamin-sensitive Ca$^{2+}$-dependent K$^+$ currents (Debarbieux et al. 1998).

Local drug injections were performed by applying air pressure pulses (0.01–0.05 s) to a patch pipette or a two-barrel pipette filled with a solution containing 200 μM GABA or 200 μM isoguvacine, a selective GABA$_A$ agonist. These drugs were dissolved either in ACSF or in a modified ACSF where NaCl had been replaced by choline chloride. The tip of the two-barrel pipette was broken under visual control to a diameter of 3 μm (or ~1-μm ID for each barrel). The ejection pipette was positioned directly above the recorded soma. The recordings were first obtained in ACSF to identify the cells firing pattern. Then, GABA or isoguvacine was applied in the presence of TTX (0.5 μM) to avoid polysynaptic phenomena. To determine whether the GABA agonists leaked from the ejection pipette, we compared the amount of spontaneous synaptic activity (quantified by computing the standard deviation of the intracellular signal) displayed by neurons recorded in the presence versus absence of the pipettes. No differences were observed.

Analyses were carried out off-line with the software IGOR (WaveMetrics) and home-made software running on Macintosh microcomputers. The input resistance ($R_{in}$) of the cells was estimated in the linear portion of current-voltage plots. The membrane time constant was derived from single-exponential fits of voltage responses to small hyperpolarizing current pulses. Statistical significance of the results was determined with Student’s $t$-tests (2-tailed). All values are expressed as means ± SE.

**Morphological identification of recorded cells**

When recorded cells were injected with Neurobiotin, the slices were removed from the chamber and fixed for 1–3 days in 0.1 M phosphate buffer saline (pH 7.4) containing 2% paraformaldehyde and 1% glutaraldehyde. Slices were then embedded in gelatin (10%) and sectioned on a vibrating microtome at a thickness of 60–100 μm. Neurobiotin-filled cells were visualized by incubating the sections in the avidin-biotin-horseradish peroxidase (HRP) solution (ABC Elite Kit, Vector Labs) and processed to reveal the HRP staining.

**RESULTS**

A total of 62 RS and 41 FS cells generating overshooting action potentials were recorded in the whole cell configuration. These recordings were obtained in the lateral amygdaloid (LA) nucleus (RS, $n = 21$; FS, $n = 13$) as well as in the perirhinal (RS, $n = 23$; FS, $n = 21$) and parietal (RS, $n = 18$; FS, $n = 7$) cortices. As similar results were obtained in these three regions, they will be considered as a group for simplicity. However, averages for each area are provided in Table 1.

As in previous studies (see references in INTRODUCTION), RS neurons were distinguished from FS cells on the basis of their repetitive firing behavior, particularly the presence (Fig. 1A1) and lack (Fig. 1B1) of spike frequency adaptation, respectively. Moreover, significant differences in resting $V_m$, $R_{in}$ time constant, and spike duration (2-tailed $t$-tests, $P < 0.05$) were also noted (see Table 1).

Moreover, consistent with findings obtained in this (Lang and Paré 1998; Martina et al. 2001; Paré et al. 1995) and other laboratories (Faulkner and Brown 1999; McCormick et al. 1985; Rainnie et al. 1993; Washburn and Moises 1992a), RS and FS cells had different morphological properties. RS cells ($n = 13$; Fig. 1A, 2 and 3) were spiny, multipolar, often pyramidal-shaped neurons, whereas FS neurons (Fig. 1B2) had aspiny, sometimes varicose dendrites (Fig. 1B3) that formed trees of various shapes ($n = 8$). Thus despite significant morphological heterogeneity among RS and FS cells, they could be distinguished unambiguously by the presence or lack of dendritic spines, respectively.

**Responses to synaptically released GABA**

To study the effect of synaptically released GABA in the absence of fast glutamatergic events, RS and FS neurons were recorded in the presence of glutamate receptor antagonists (CNQX, 20 μM; AP-5, 100 μM) at proximity of tungsten stimulating electrodes (Fig. 2).

**RS NEURONS.** Consistent with previous in vitro findings in other cortical regions, basolateral amygdaloid nuclei and species (Dutar and Nicoll 1988; McCormick 1989; Rainnie et al. 1991; Scanziani et al. 1991; Washburn and Moises 1992b), electrical stimuli elicited a biphasic inhibitory postsynaptic potential (IPSP) in RS neurons (Fig. 2A1). Its early (●) and late (+) components reversed at significantly different potentials.
TABLE 1. Physiological properties and GABA<sub>A</sub> reversals of RS and FS neurons

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Resting V&lt;sub&gt;ir&lt;/sub&gt;, mV</th>
<th>R&lt;sub&gt;ir&lt;/sub&gt;, MΩ</th>
<th>τ&lt;sub&gt;r&lt;/sub&gt;, ms</th>
<th>Spike amplitude, mV</th>
<th>Spike duration at half-amplitude, ms</th>
<th>GABA&lt;sub&gt;A&lt;/sub&gt; Reversal</th>
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<td></td>
<td>Endogenous&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Regular spiking</td>
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<tr>
<td>LA</td>
<td>-82.2 ± 1.0</td>
<td>198.5 ± 18.5</td>
<td>32.6 ± 3.2</td>
<td>80.7 ± 1.7</td>
<td>1.24 ± 0.08</td>
<td>-74.1 ± 1.9</td>
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<tr>
<td>n</td>
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<tr>
<td>PRH</td>
<td>-81.0 ± 1.4</td>
<td>183.0 ± 12.3</td>
<td>32.1 ± 2.4</td>
<td>85.3 ± 2.1</td>
<td>1.15 ± 0.04</td>
<td>-70.0 ± 1.0</td>
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<tr>
<td>Parietal</td>
<td>-80.6 ± 1.0</td>
<td>235.2 ± 19.5</td>
<td>29.9 ± 1.2</td>
<td>82.4 ± 1.3</td>
<td>1.10 ± 0.04</td>
<td>-74.6 ± 1.8</td>
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<tr>
<td>Pooled</td>
<td>-81.3 ± 0.6</td>
<td>203.7 ± 9.8</td>
<td>31.3 ± 1.5</td>
<td>82.9 ± 1.1</td>
<td>1.16 ± 0.03</td>
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<tr>
<td>LA</td>
<td>-66.4 ± 1.6</td>
<td>427.1 ± 45.7</td>
<td>21.4 ± 2.6</td>
<td>86.2 ± 3.1</td>
<td>0.56 ± 0.04</td>
<td>-54.8 ± 1.1</td>
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<td>PRH</td>
<td>-68.0 ± 1.2</td>
<td>352.8 ± 43.7</td>
<td>17.1 ± 2.1</td>
<td>81.3 ± 4.6</td>
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<td>Parietal</td>
<td>-69.2 ± 1.6</td>
<td>395.1 ± 43.6</td>
<td>19.2 ± 3.5</td>
<td>87.4 ± 1.8</td>
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<tr>
<td>Pooled</td>
<td>-67.7 ± 0.8</td>
<td>384.3 ± 27.2</td>
<td>19.1 ± 1.5</td>
<td>84.0 ± 2.6</td>
<td>0.50 ± 0.03</td>
<td>-54.1 ± 1.3</td>
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<td>n</td>
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Values are means ± SE. *Electrically evoked inhibitory postsynaptic potential in the presence of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and (−)-2-amino-5-phosphonopentanoic acid (AP-5). For regular-spiking (RS) cells, responses to isoguvacine. For fast-spiking (FS) cells, results obtained with isoguvacine and GABA were pooled.

FS NEURONS. In contrast with the CNQX- and AP-5-resistant potentials evoked in RS cells, those observed in FS neurons were monophasic (Fig. 2B1), had a shorter duration (128 ± (paired t-test, P < 0.05; n = 12) of −72.4 ± 1.03 and −90.2 ± 2.17 mV (Fig. 2A2), thus suggesting that they were mediated by a Cl<sup>-</sup> (GABA<sub>A</sub>) and a K<sup>+</sup> (GABA<sub>B</sub>) conductance, respectively (see references in the preceding text). Consistent with this, addition of the GABA<sub>A</sub> receptor antagonist bicuculline (10 μM) markedly reduced the early phase of the IPSP (Fig. 2A3; reduction of 85 ± 4.1%; n = 10 at −90 mV; paired t-test, P < 0.05) but did not diminish the late one. Conversely, bath application of the GABA<sub>B</sub> antagonist saclofen (100 μM, n = 4) reduced the late IPSP by 46 ± 4.9% (paired t-tests, P > 0.05; not shown).
12.1- and 663 ± 38.1-ms delay to 95% amplitude decrement for FS and RS cell at −65 mV; t-tests, P < 0.05; n = 10 and 12, respectively), and reversed at a significantly more depolarized $V_{P}$ (Fig. 2B2; −54.1 ± 1.33 mV; t-tests, P < 0.05). In fact, the reversal potential was so close to spike threshold that it often had to be extrapolated to avoid contamination by spike afterpotentials (Fig. 2B2). Subsequent addition of bicuculline (10 μM, n = 6) or picrotoxin (100 μM, n = 4) to the perfusate, abolished CNQX- and AP-5-resistant potentials (Fig. 2B3). It is worth stressing that the effects of GABA A antagonists were tested in FS cells of the LA (n = 3), PRH area (n = 4), and parietal cortex (n = 3) and that identical results were obtained. Furthermore, in all tested cells, increasing stimulation intensities never elicited a picrotoxin- or bicuculline-resistant IPSP in FS cells.

Figure 3A illustrates the GABA A reversal (x axis) of RS (+) and FS (○) neurons recorded in the LA, PRH area, and parietal cortex (right axis). Note that regional differences in GABA A reversals were small compared to those existing between RS and FS neurons. This can also be observed in Table 1, which lists average data for each region taken individually. In this context, it should be mentioned that there are precedents in the literature for the relatively depolarized GABA A reversals of FS neurons in the cortex and LA (for instance, see Galarreta and Hestrin 1999; Lang and Paré 1998).

As an additional control, GABA A reversals were estimated in voltage-clamp mode in four RS (Fig. 4A1) and four FS (Fig. 4B1) neurons of the perirhinal cortex. Consistent with our current-clamp recordings, $E_{GABA A}$ averaged −71.5 ± 1.4 in RS cells compared to −54.6 ± 2.1 in FS cells.

![Diagram of GABA A reversals](http://jn.physiology.org/)

**FIG. 3.** Contrasting $E_{GABA A}$ of RS (+) and FS (○) cells to (A) synaptic release GABA or (B) exogenous GABA A agonists in the parietal neocortex (Par) perirhinal (PRH) area, and lateral amygdala (LA). A: responses were evoked by electrical stimuli delivered at proximity of recorded cells in the presence of CNQX and AP-5. B: GABA A agonists (RS, isoguvacine; FS, isoguvacine or GABA) were pressure applied through a patch pipette positioned above the recorded soma.

The results described above suggested that the GABA responses of RS and FS cells differ in at least two respects. First, the reversal of GABA A responses was ≈18 mV more positive in FS than RS cells. Second, Cl⁻ (GABA A) and K⁺ (GABA B) components contributed to the responses of RS cells, whereas no K⁺ component was discernible from somatic recordings in FS cells. However, since the small amplitude of CNQX- and AP-5-resistant responses might have prevented detection of a GABA B component in FS cells, we next examined the effect of local pressure application of isoguvacine via a patch pipette positioned above the recorded cells.

In keeping with the above, the responses of FS cells to exogenous GABA in the presence of TTX (0.5 μM) were completely abolished by picrotoxin (100 μM; n = 3) or by bicuculline (10 μM; n = 7; Fig. 5B) whereas those of RS neurons (n = 11) included a bicuculline and picrotoxin resistant component (Fig. 5A). Identical results were obtained in FS cells of the LA (n = 4), perirhinal area (n = 4), and parietal cortex (n = 2).

When measuring the reversal potential of GABA A responses evoked by exogenous agonists, it is important to take into account the fact that, in principal neurons at least, $E_{GABA A}$ shifts positively during prolonged GABA A IPSPs (reviewed in Kaila 1994). In part, this change occurs because the chloride gradient collapses, revealing the contribution of a bicarbonate conductance to GABA A responses (reviewed in Voipio and Kaila 2000). As a result, the initial negative potential caused by GABA A activation decays, giving rise to a positive potential. Moreover, with long agonist applications, nonsynaptic factors such as K⁺ release further contaminate later response compo-
ments (Voipio and Kaila 2000). As a result, responses to pro-
longed GABA_A activation are multiphasic (see Figs. 5 and 6).

Because our objective was to compare the GABA responses of
FS and RS cells in basal conditions, we first investigated how
E_{GABA_A} changed over time. The goal of this analysis was
to determine an optimal interval to measure GABA_A reversals
before the occurrence of significant changes in intracellular
chloride concentration.

Figure 6 shows the responses of representative RS and FS
neurons to the pure GABA_A agonist isoguvacine (200 μM).
Note that these responses are much longer than those evoked
by GABA, presumably because agonist diffusion rather than
re-uptake (Schousboe 2000) is the main factor terminating
these responses. GABA_A reversals were computed using the
approach shown in Fig. 6, A2 and B2, but at various intervals
increasing initially in steps of 10 ms. As shown in Fig. 6C for
RS (○, n = 9) and FS (●, n = 7) neurons, measurements
obtained ≤20 ms after response onset yielded variable results
(note larger SE values), probably because response amplitudes
were still low. Intercell variability reached acceptable levels 30
ms after response onset, and this interval also yielded the most
negative GABA_A reversals.

Later on, E_{GABA_A} shifted positively, but much more in RS
than FS cells (shift of 8.7 ± 0.5 and 1.3 ± 0.7 mV between
0.03 and 1 s, respectively). Note that the positive shifts in
E_{GABA_A} reached statistical significance in both cell types
(paired 2-tailed t-tests at P < 0.05). Thus in the following
account, E_{GABA_A} was measured 30 ms after response onset
when it was most negative in both cell types.

Consistent with the results obtained with electrical stimuli,
isoguvacine-evoked responses reversed at −57.1 ± 1.2 mV in
FS cells (n = 7; Fig. 6B2). Moreover, similar results were
obtained with GABA (−57.6 ± 0.6 mV, n = 15). As shown in
Fig. 3B, negligible differences in E_{GABA_A} were seen between
FS cells of the LA (n = 6), perirhinal area (n = 13), and
parietal cortex (n = 3; see Fig. 3B and individual averages in
the right-most column of Table 1).

To determine E_{GABA_A} in RS neurons, we only considered
responses to pressure-applied isoguvacine. Despite the absence
of a GABA_B component in these responses, their reversal
potential remained significantly more negative than that of FS
neurons (−72.8 ± 0.6; n = 50; unpaired t-tests, P < 0.05; Fig.
6A2), whether the recordings were obtained in the perirhinal
area (n = 17), parietal cortex (n = 15), or LA (n = 18; see Fig.
3B and Table 1, right-most column).

As an additional control, the reversal potential of re-
sponses evoked by local pressure application of isoguvacine
was estimated in voltage-clamp mode in four RS (Fig. 4A2)
and four FS (Fig. 4B2) neurons of the perirhinal cortex.
Consistent with our current-clamp recordings, E_{GABA_A}
averaged −72.8 ± 2.3 in RS cells compared to −56.9 ± 2.9
in FS neurons.

Note that while we cannot exclude the possibility of space-
clamp errors, it is unlikely that they completely account for the
difference in E_{GABA_A}. Indeed, in the absence of TTX, GABA
and isoguvacine could evoke one or more action potentials in
FS cells at rest but not in RS neurons. It is likely that the more
positive GABA_A reversal of FS cells compared to RS neurons
accounts for this.

E_{GABA_A} in gramicidin perforated-patch recordings

It is conceivable that the differences in E_{GABA_A} resulted
from the fact that FS cells tend to have a smaller volume than
RS neurons, thus causing a more rapid and/or complete dialysis
of FS cells by the pipette solution. However, this appears
unlikely because E_{GABA_A} of FS and RS neurons differed from
the calculated E_{Cl} (−61.4 mV with our solutions), but in
opposite directions. Nevertheless, we tested this using perfo-
rated-patch recordings of RS (n = 7) and FS (n = 6) cells with
the cation-selective ionophore gramicidin (Ebihara et al. 1995;
Kyrozis and Reichling 1995; Myers and Haydon 1972). In
disagreement with the possibility that a differential dialysis of
FS and RS neurons accounted for our results, an even greater
difference in E_{GABA_A} was found in this recording configuration
(RS, −75.2 ± 1.3 mV; FS, −51.9 ± 1.9 mV; unpaired t-tests,
P < 0.05).

Examples of isoguvacine-evoked responses are shown for a
RS (Fig. 7A) and a FS cell (Fig. 7B) recorded in the perforated-
patch configuration (top) and, after rupture of the membrane, in
the whole cell mode (bottom). In both cells, rupturing the
membrane produced a large positive shift in E_{GABA_A} (note
different voltage calibrations in Fig. 7, top and bottom) because
a pipette solution with a high intracellular chloride concentra-
tion was used to monitor the state of the membrane in these
experiments.
reduced (Kakazu et al. 1999; Rohrbough and Spitzer 1996), a negative shift of \( E_{\text{GABA}_A} \) in low \([\text{Na}^+]_o \) would be consistent with the presence of a NKCC.

\( E_{\text{GABA}_A} \) of RS cells \((n = 13)\) was not changed significantly in low \([\text{Na}^+]_o \), suggesting that NKCC plays a minor role in this cell type. In contrast, reducing \([\text{Na}^+]_o \) reversibly shifted \( E_{\text{GABA}_A} \) by \(-5.2 \pm 1.2\) mV in FS cells \((n = 10); \text{paired} t\)-test, \( P < 0.05 \). However, it is also possible that reducing \([\text{Na}^+]_o \), interfered with \( \text{Na}^+\)-dependent acid extrusion (Romero and Boron 1999). Thus we tested the effect of adding bumetanide (20 \( \mu \)M), a selective NKCC inhibitor, to the perfusate (Van Aubel et al. 2000).

Consistent with the idea that FS but not RS cells are endowed with a NKCC, bumetanide hyperpolarized \( E_{\text{GABA}_A} \) in FS cells (Fig. 8A, ○; by \(-5.1 \pm 1.1\) mV after 20 min; \( n = 7 \); paired \( t\)-test, \( P < 0.05 \)), but it had no effect on RS neurons (Fig. 8A, ●). Moreover, in the presence of bumetanide, addition of furosemide (1 \( \mu \)M), a nonspecific inhibitor of cation-chloride cotransporters (Van Aubel et al. 2000) did not change \( E_{\text{GABA}_A} \) in FS cells (Fig. 8B, ○) but depolarized it by \( 7.5 \pm 0.4\) mV in RS neurons (Fig. 8B, ●; \( n = 6 \); paired \( t\)-test, \( P < 0.05 \)). This suggests that KCC is the predominant regulator of \( E_{\text{Cl}^-} \) in RS neurons but not in FS cells.

**DISCUSSION**

Our results suggest that in the basolateral amygdala as well as in the parietal and perirhinal cortices: synaptically released

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**FIG. 6.** Responses of RS (A) and FS (B) neurons to isoguvacine, pressure applied through a patch pipette positioned above the recorded soma. TTX was present throughout. 1: responses to isoguvacine (isog.) evoked from different \( V_m \) (numbers on the left, mV) as determined by intracellular current injection. 2: graph plotting response amplitude as a function of \( V_m \). Measurements were made at the time points indicated by ↑. The neurons in A and B were recorded in the perirhinal and parietal cortices, respectively. C: graph plotting the reversal potential of isoguvacine-evoked responses (●, RS, \( n = 9 \); ○, FS, \( n = 7 \)) as a function of time from response onset. The reversal potentials were determined as in A2 and B2. Note that irrespective of the interval between response onset and reversal measurements, \( E_{\text{GABA}_A} \) of RS and FS cells remained different.

**Effect of extracellular Na\(^+\), bumetanide, and furosemide on GABA\(_A\) reversals**

Because the main permeant ion of GABA\(_A\) responses is chloride (Bormann et al. 1987; Kaila 1994; Kaila and Voipio 1987; Kaila et al. 1989), the preceding led us to suspect that RS and FS cells are endowed with different chloride homeostatic mechanisms. Indeed, previous work has revealed that various factors determine the transmembrane chloride gradient (Kaila and Voipio 1987) including a potassium-chloride cotransporter (KCC)-mediating chloride extrusion (Misgeld et al. 1986; Thompson et al. 1988) and Na-K-2Cl cotransporter (NKCC) responsible for chloride uptake (Kakazu et al. 1999; Rohrbough and Spitzer 1996).

To test the possibility that the more depolarized \( E_{\text{GABA}_A} \) of FS cells compared with RS neurons reflects the differential action of a NKCC, we first examined the effects of reducing the extracellular \( \text{Na}^+ \) concentration \([\text{Na}^+]_o \) from 153 to 27 mM (by equimolar replacement of NaCl with choline chloride). Because NKCC activity diminishes when \([\text{Na}^+]_o \) is

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**FIG. 7.** Responses of RS (A) and FS (B) neurons to pressure-applied isoguvacine. The same cells were recorded with the perforated-patch method (1) and after rupturing the membrane by gentle suction (2). The pipette contained a high-chloride concentration, thus explaining the large shift in \( E_{\text{GABA}_A} \) from 1 to 2. The neurons in A and B were recorded in the parietal and perirhinal cortices, respectively. Voltage calibration in A1 applies to B1. Same time base in 1 and 2. Isog, isoguvacine.
and exogenous GABA evoke GABA_A IPSPs in FS interneurons, but biphasic GABA_A-B IPSPs in RS cells; $E_{\text{GABA}_B}$ is much closer to threshold in FS than RS neurons; and this difference arises from cell-type-specific chloride homeostatic mechanisms whereby the prevalent regulators of $[\text{Cl}^-]$ are cation-chloride cotransporters that accumulate chloride in FS cells and extrude chloride in RS neurons.

**Are principal cells and fast-spiking interneurons endowed with different complements of GABA receptors in their somatodendritic compartment?**

While our observations on the effect of GABA in RS cells are consistent with previous findings (Dutar and Nicoll 1988; McCormick 1989; Rainnie et al. 1991; Scanziani et al. 1991; McCormick 1989; Rainnie et al. 1991; Scanziani et al. 1991; Washburn and Moises 1992b), the complete abolition of GABA responses by picrotoxin in FS cells is surprising. Indeed, this result suggests that FS cells lack functional GABA_B receptors in their somatodendritic compartment or that, if present, they are not coupled to the inwardly rectifying K^+ conductance that typically mediates postsynaptic GABA_B responses (Gähwiler and Brown 1985).

In the hippocampus, some interneurons do generate GABA_B responses. For instance, this is the case of interneurons located in the CA1 pyramidal layer (Lacaille 1991) but not those located in stratum oriens (Morin et al. 1996). Conflicting results were obtained in stratum radiatum interneurons (Morin et al. 1996; Verheugen et al. 1999).

Could the lack of GABA_B responses in our FS cells be due to dialysis of the cells interior by the pipette solution? This appears unlikely because RS neurons recorded with the same method displayed large GABA_B IPSPs. Another possibility, namely that an insufficient amount of GABA was released to activate GABA_B IPSPs, seems improbable because high-intensity stimuli applied in control medium also failed to elicit GABA_B responses (unpublished observations). Moreover, the responses of FS cells to large pressure applications of GABA were completely abolished by GABA_A antagonists.

Our observations are at odds with other studies indicating that the axon terminals of at least some types of GABAergic interneurons express GABA_B receptors (Misgeld et al. 1995). However, it is possible that GABA_B receptors and/or their G-protein-coupled effectors are expressed in a compartment-

**Cell-type-specific chloride homeostasis in FS and RS neurons**

In this study, the differing GABA_A reversals of RS and FS neurons were observed with synaptically released GABA as well as during the early part of responses evoked by exogenous GABA_A agonists. This rules out the involvement of nonsynaptic factors such as K^+ release (Voipio and Kaila 2000) or the bicarbonate-induced chloride uptake that occurs during prolonged GABA responses (Kaila 1994; Kaila and Voipio 1987; Kaila et al. 1989). In this context, it appears unlikely that GABA_A agonists produced a faster reduction of the chloride gradient in FS cells because they have a smaller volume than RS neurons. Indeed, using the same methods, $E_{\text{GABA}_B}$ was $-70 \text{ mV}$ in intercalated amygdala neurons, one of the smallest types of neurons in the brain (Royer et al. 1999).

Another possibility is that our local GABA applications affected different cellular compartments in FS versus RS cells. This is important because some cell types were reported to exhibit compartment-specific chloride gradients (Andersen et al. 1980; Misgeld et al. 1986; however, see van Brederode et al. 2001). In addition, if GABA_A receptors were located at different electrotonic distances in FS and RS cells, differences in GABA_A reversal could be ascribed to a space-clamp problem. Unfortunately, although our local GABA injections were performed directly at the soma level, we cannot rule out a preferential expression of GABA_A receptors in the dendrites of FS cells.

In light of our findings, a more likely explanation is that RS and FS neurons are endowed with different intracellular chloride homeostatic mechanisms. Indeed, chloride is the main permeant ion of GABA_A receptors (Bormann et al. 1987; Kaila and Voipio 1987; Kaila et al. 1989). Thus, its differential distribution across the membrane should largely determine $E_{\text{GABA}_B}$. Bicarbonate, whose permeability through GABA_A channels is $\sim 0.2$–0.4 of that of chloride (Bormann et al. 1987; Kaila and Voipio 1987; Kaila et al. 1989), probably produced a slight positive shift of $E_{\text{GABA}_B}$ in our recording conditions.

In support of the hypothesis that RS and FS neurons are endowed with different intracellular chloride homeostatic mechanisms, pharmacological inhibition of NKCC hyperpolarized $E_{\text{GABA}_B}$ in FS cells but had no effect in RS neurons. In contrast, blocking KCC depolarized $E_{\text{GABA}_B}$ in RS neurons, leaving it unchanged in FS cells. It should be pointed out that such cell-type-specific regulation of $[\text{Cl}^-]$, by cation-chloride cotransporters is not unique to the cerebral cortex and basolateral amygdala; it was observed previously in spinal neurons of Xenopus larvae (Rohrbough and Spitzer 1996) as well as in the retina (Vardi et al. 2000) and the thalamus (Ulrich and Huguenard 1997).

While the presence of NKCC in FS cells of the cortex and amygdala was unknown, it had been reported that a KCC cotransporter actively extrudes chloride in principal cortical neurons (Misgeld et al. 1986; Thompson et al. 1988). In fact, the postnatal development of KCC (Kakazu et al. 1999; Rivera et al. 1999) underlies the shift from depolarizing to hyperpolarizing GABA_A responses during neuronal maturation (Luhmann and Prince 1991).
Incidentally, the presence of hyperpolarizing GABA_A responses in RS cells suggests that our preparation was mature. Although it is conceivable that GABA responses mature later in FS than RS neurons, this possibility seems remote given that, in the hippocampus, interneurons form mature synapses earlier than principal cells (Tizio et al. 1999).

**Implications for neuronal excitability and synchronization**

Recently, the role of interneurons in synchronizing distributed populations of pyramidal cell has been emphasized (Traub et al. 1998). In the hippocampus and neocortex, it was proposed that the divergent projections of inhibitory interneurons to principal cells play a critical role in generating fast synchronized oscillations (Buhl et al. 1998; Buzsáki and Chrobak 1995; Cobb et al. 1995; Fisahn et al. 1998; Tamás et al. 2000; Traub et al. 1996). Indeed, interneurons are coupled by chemical synapses (Somogyi et al. 1998; Tamás et al. 1998) and gap junctions (Galarreta and Hestrin 1999; Gibson et al. 1999). As a result, in conditions of affenter excitation, interneurons would generate synchronized IPSPs in thousands of pyramidal cells, thus entraining them to fire preferentially on the decaying phase of IPSPs, in phase with the local field potential (Buhl et al. 1998; Buzsáki and Chrobak 1995; Cobb et al. 1995; Fisahn et al. 1998; Tamás et al. 2000; Traub et al. 1996).

Our results imply that rhythmic GABA IPSPs should have a different impact on FS interneurons and principal cells because $E_{\text{GABA}}$ is much closer to spike threshold in FS cells. As a result, during periods of synchronized network activity, when neurons are depolarized to near-threshold levels, GABA inhibition should produce a transient decrease in $R_m$ with little $V_m$ change in FS cells. Thus, these neurons should be more readily available for synaptic recruitment on a cycle-to-cycle basis than RS neurons. In agreement with this, FS cells were reported to fire in a higher proportion of cycles than principal cells during fast oscillations (Penttonen et al. 1998).

This work was supported by the Canadian Institutes of Health Research, the Natural Sciences and Engineering Research Council, and the National Institute of Neurological Disorders and Stroke (R01-NS-37711).

**NOTE ADDED IN PROOF**

Recently, it was reported that the KCl cotransporter, KCC2, is highly expressed in the vicinity of excitatory synapses in pyramidal cells (Gulyás et al. 2001). Although these findings seem to contradict our results, the discrepancy might only be apparent. For instance, it is possible that KCC2 cannot compensate for the chloride load generated by other cotransporters, particularly NKCC1.

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