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Postnatal Development of Hippocampal Dentate Granule Cell γ-Aminobutyric Acid$_A$ Receptor Pharmacological Properties

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ABSTRACT

Postnatal development of hippocampal dentate granule cell γ-aminobutyric acid$_A$ (GABA$_A$) receptor pharmacological properties was studied. Granule cells were acutely isolated from hippocampi of 7- to 14- and 45- to 52-day-old rats, and whole cell patch-clamp recordings were obtained. The sensitivity of GABA$_A$ receptors to GABA and modulation of GABA$_A$ receptor currents by benzodiazepines (BZ), zinc, furosemide, and loreclezole was studied. Multiple changes in the pharmacological properties of dentate granule-cell GABA$_A$ receptors occurred during the first 52 days of postnatal development: GABA-evoked maximal current increased with postnatal age; GABA$_A$ receptors changed from BZ type 3 in young rats to BZ type 1 in adult rats; furosemide and zinc inhibited GABA$_A$ receptor currents in young rats but not in adult rats; the fraction of cells that expressed loreclezole-sensitive GABA$_A$ receptors increased with postnatal age. These findings suggest that dentate granule cells in young and adult animals express pharmacologically distinct GABA$_A$ receptors and that the postnatal development of these receptors is prolonged, lasting at least 45 days. Comparison with the previously reported pharmacological properties of GABA$_A$ receptors on dentate granule cells acutely isolated from hippocampi of 28- to 35-day-old rats suggests that receptors expressed at that age have properties intermediate between young and adult rats.

γ-Aminobutyric acid (GABA) plays several trophic roles during development, including stimulation of outgrowth of neuronal processes (Behar et al., 1996), modulation of DNA synthesis (LoTurco et al., 1995), and regulation of neuronal phenotype and depolarization of immature neurons (Ben-Ari et al., 1994). The sequence of changes that result in transformation of GABA from a trophic (Behar et al., 1996), excitatory neurotransmitter (Ben-Ari et al., 1994; Ben-Ari et al., 1997) in the immature brain to the major inhibitory neurotransmitter in the forebrain involves changes in chloride ion reversal potential, changes in expression and distribution of glutamic acid decarboxylase 67 and 65 (Dupuy and Houser, 1996), and late coupling of GABA$_B$ receptors to potassium channels (Ben-Ari et al., 1997). There is growing evidence that this transformation also may involve developmental changes in the subunit subtype composition and properties of GABA$_A$ receptors.

The GABA$_A$ receptor is a pentameric subunit complex that contains specific binding sites for GABA and multiple allosteric regulators, including picrotoxin, barbiturates, benzodiazepines (BZs), zinc, and the anesthetic steroids, and that forms a chloride ion channel (Macdonald and Olsen, 1994). Based on sequence similarity, six different GABA$_A$ receptor subunit families have been identified in mammals (α, β, γ, δ, ϵ, and π) (Macdonald and Olsen, 1994; Davies et al., 1997; Whiting et al., 1997). Several of the subunit families have multiple subtypes (α1–α6, β1–β3, and γ1–γ3). Marked changes occur in the expression of GABA$_A$ receptor subunit subtype mRNAs and receptor polypeptides and functional receptor properties during development (Laurie et al., 1992; Fritschy et al., 1994; Mathews et al., 1994; Thompson et al., 1996). Because the subunit composition of GABA$_A$ receptors determines their pharmacological properties, it is likely that the pharmacological properties of GABA$_A$ receptors change during development.

Granule cells of the hippocampal dentate gyrus provide a useful system in which to study GABA$_A$ receptor development because many granule cells are born, proliferate, migrate, and mature in the postnatal period (Altman and Das, 1965; Altman and Das, 1966; Gould and Cameron, 1996). A recent study in dentate granule cells (Hollrigel and Soltesz, 1997) demonstrated that until the end of the second postnatal week, synaptic GABA$_A$ receptor-mediated miniature inhibitory postsynaptic currents (mIPSC) displayed slower rise and decay kinetics than those of adult granule cells. It was proposed that these developmental changes in mIPSC kinet-

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ABBREVIATIONS: GABA, γ-aminobutyric acid; IPSC, inhibitory postsynaptic current; PIPES, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); IC$_{50}$, 50% inhibitory concentration; BZ, benzodiazepine.

ics reflected postnatal development of the properties of the $GABA_A$ receptors.

We report the postnatal development of the properties of $GABA_A$ receptors on dentate granule cells from 7 to 52 days of age. Pharmacological properties of $GABA_A$ receptors on dentate granule cells acutely isolated from hippocampi of young rats (7–14 days) and adult rats (45–52 days) were characterized. In the past, we have characterized the pharmacological properties of $GABA_A$ receptors present on dentate granule cells acutely isolated from 28- to 35-day-old rats (Kapur and Macdonald, 1996); the pharmacological properties of $GABA_A$ receptors in these three age groups were compared.

Materials and Methods

Cell Isolation. Dentate granule cells were isolated from rats aged between 7 and 52 days according to the method described originally by Kay and Wong (1986) and later modified (Oh et al., 1995). The brain was dissected free, and the region containing the hippocampus was blocked and chilled in an oxygenated piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES) -buffered medium (4°C) for 1 min. The PIPES buffer solution contained 120 mM NaCl, 2.5 mM KCl, 1.5 mM $CaCl_2$, 1 mM $MgCl_2$, 25 mM D-glucose, and 20 mM PIPES, pH 7.0. After blot-drying, the brain was mounted on a vibratome stage, and 500-μm coronal sections containing the hippocampus were cut. The sections were allowed to recover in oxygenated (95% O_2/5% CO_2) PIPES buffer for 30 to 60 min. Hippocampal sections were then incubated in oxygenated SIGMA type XXIII protease enzyme (Sigma Chemical Company, St. Louis, MO) in the buffer at 32°C for 30 to 45 min. The dentate gyrus was dissected out and cut into 0.5-mm cubes that were triturated in a cold (4°C) PIPES-buffered medium in fire-polished glass pipettes to isolate neurons. The isolated neurons were plated on poly-L-lysine-coated, 35-mm, polystyrene Petri dishes (Corning Glass Works, Corning, NY), and the recordings were made within 1 h of isolation.

Whole Cell Recording. Whole cell $GABA_A$ receptor currents were recorded from hippocampal dentate granule cells acutely isolated from 7- to 52-day-old rats using the technique described by Hamill et al., (1981). The extracellular recording solution consisted of 142 mM NaCl, 1.0 mM $CaCl_2$, 8 mM KCl, 6 mM $MgCl_2$, 10 mM glucose, and 10 mM HEPES, pH adjusted to 7.4 and osmolarity of 310 to 320 mOsM (all reagents from Sigma). Glass recording patch pipettes were filled with a solution consisting of 115 mM dibasic Trizma phosphate, 30 mM Trizma base, 11 mM EGTA, 2 mM $MgCl_2$, and 0.5 mM $CaCl_2$, pH 7.35. Recording pipettes also contained ATP (2 mM) unless otherwise specified. All recordings were obtained at room temperature (24°C). With a bathing solution containing a chloride concentration of 164 mM and whole cell recording pipettes containing a 5 mM chloride ion solution, the chloride ion concentration gradient produced a chloride ion equilibrium potential (E_{Cl^-}) of −76 mV. Granule cells were voltage-clamped to 0 mV; thus, application of GABA produced outward currents. Patch pipettes (resistance of 6–10 MΩ) were pulled on P-87 Flaming Brown puller by a four-stage pull. Currents were recorded with an Axopatch 200 A amplifier (Axon Instruments, Foster City, CA) and low-pass filtered at 2 kHz with an eight-pole Bessel filter (Frequency Devices, Haverhill, MA) before digitization, storage, and display. Currents were displayed on a Gould 2400S chart recorder, and peak whole cell currents were measured manually from the chart paper. Currents were also recorded on a hard disk using the Axotape or Axoscope program (digitized at 208 Hz) and on a video cassette tape recorder (Sony SL-HF360) via a digital audio processor (Sony PCM-501 ES, 14-bit, 44 kHz).

Drug Application. GABA, zolpidem, and $ZnCl_2$ dissolved in extracellular solution were applied to neurons using a modified U-tube "multipuffer" rapid application system (Greenfield and Macdonald, 1996), with the tip of application pipette placed 100 to 200 μm from the cell. Diazepam and furosemide were dissolved first in dimethyl sulfoxide and then diluted in extracellular buffer; the final dimethyl sulfoxide dilution was at least 1:50,000. GABA, diazepam, and $ZnCl_2$ were obtained from Sigma. Zolpidem was obtained from Research Biochemicals, Inc. (Natick, MA).

Data Analysis. The magnitude of the enhancement or inhibition of $GABA_A$ receptor current by a drug was determined by dividing the peak amplitude of $GABA_A$ receptor current elicited in the presence of a given concentration of the drug and GABA by the peak amplitude of control current elicited by GABA alone and multiplying the fraction by 100 to express it as percentage control. The control response was 100%. Peak $GABA_A$ receptor currents at various drug concentrations were fitted to a sigmoidal function using a four-parameter logistic equation (sigmoidal concentration-response) with a variable slope. The equation used to fit the concentration-response relationship was

$$I = \frac{I_{max}}{1 + 10^{(\text{Log EC}_{50} - \log [\text{drug}]) * \text{HillSlope}}}$$

where I is the $GABA_A$ receptor current at a given GABA concentration, and I_{max} is the maximal $GABA_A$ receptor current. Maximal current and concentration-response curves were obtained after pooling data from all neurons tested for GABA and for all drugs. The curve-fitting algorithm minimized the sum of the squares of the actual distance of points from the curve. Convergence was reached when two consecutive iterations changed the sum of squares by less than 0.01%. The curve fit was performed on an IBM PC-compatible personal computer using the program Prism (Graph Pad Software, Inc., San Diego, CA). All data is presented as mean ± S.E.M..

Results

Maximal $GABA_A$ Receptor Current Amplitude Increased During Development. GABA at concentrations ranging from 0.3 to 1000 μM was applied to granule cells isolated from 7- to 14-day-old rats with recovery intervals of at least 1 min (Fig. 1A). In these cells, the minimum concentration of GABA required for evoking currents was 1 μM, and the peak current elicited by 10 μM GABA was 40 ± 10 pA ($n = 7$). In contrast, GABA evoked larger currents from granule cells isolated from 45- to 52-day-old rats (Fig. 1B). In these cells, the minimum concentration of GABA required for evoking currents was 0.3 μM, and the peak current elicited by 10 μM GABA was 252 ± 64 pA ($n = 8$, $p < .01$, unpaired t test) (Fig. 1B). GABA concentration-response curves were obtained from individual granule cells isolated from 7- to 14- ($n = 7$) and 45- to 52-day-old rats ($n = 8$) (Fig. 2) for GABA concentrations ranging from 1 to 1000 μM. The maximal $GABA_A$ receptor current increased with age. Maximal $GABA_A$ receptor current elicited from granule cells isolated from younger rats was 476 ± 65 pA ($n = 8$), whereas maximal current elicited from granule cells isolated from older rats was 893 ± 160 pA ($n = 7$, $p < .01$, unpaired t test) (Figs. 1 and 2). GABA potency did not change significantly during this developmental period; the GABA EC_{50} value for granule cells from younger rats was 40 ± 8 μM, whereas the GABA EC_{50} value for granule cells from older rats was 31 ± 10 μM ($p > .05$) (Fig. 2).

BZ Sensitivity of Granule Cell $GABA_A$ Receptors Increased During Development. Diazepam (100 nM) was coapplied with 10 μM GABA to granule cells isolated from 7- to 14-day-old rats and compared with peak currents elicited

by GABA alone. There was minimal enhancement of peak currents by 100 nM diazepam [12 ± 8% larger than control currents (n = 3)] (Fig. 3A). In contrast, in granule cells isolated from 45- to 52-day-old rats, 100 nM diazepam enhanced $GABA_A$ receptor currents elicited by 10 μM GABA by 53 ± 13%, ($p < .01$, n = 4, ANOVA with post-test Newman-Keuls multiple comparison test, compared with enhancement by 100 or 1000 nM diazepam in cells from 7- to 14-day-old rats) (Fig. 3B).

The larger enhancement elicited by diazepam in 45- to 52-day-old rats could have resulted from changes in the either potency or efficacy of diazepam in enhancing the $GABA_A$ receptor currents. These possibilities were evaluated by obtaining diazepam concentration-$GABA_A$ receptor current enhancement (response) curves in granule cells acutely isolated from 7- to 14-day-old rats and 45- to 52-day-old rats (Fig. 3C). In granule cells acutely isolated from 7- to 14-day-old rats, the data were fit poorly by a sigmoidal function, largely because a plateau of maximal enhancement could not be obtained [application of high diazepam concentrations (>1 μM) inhibited $GABA_A$ receptor currents]. In the fit of data from the younger rats, the diazepam EC_{50} value was 600 nM, and the maximal enhancement of $GABA_A$ receptor currents was 34 ± 3%. In older rats, the diazepam EC_{50} value was 24 ± 7 nM, and the maximal enhancement of $GABA_A$ receptor currents was 53 ± 10%. Thus $GABA_A$ receptors present on granule cells isolated from older rats had higher apparent diazepam affinity for $GABA_A$ receptor currents.

The BZ receptor pharmacology of dentate granule-cell $GABA_A$ receptors present on 7- to 14-day-old and 45- to 52-day-old rats was further characterized. BZ receptor sites have been designated types BZ 1, BZ 2, or BZ 3 based on high, low, or no affinity, respectively, for certain BZ agonists such as zolpidem (Sieghart and Drexler, 1983; Wieland et al., 1992). BZ 1 receptors have high zolpidem affinity whereas BZ 2 receptors have low zolpidem affinity. $GABA_A$ receptor currents in dentate granule cells from 7- to 14-day-old rats were relatively zolpidem-insensitive, being enhanced only 10 to 15% by high concentrations (100 nM to 1 μM) of zolpidem (Fig. 4A). $GABA_A$ receptors on dentate granule cells (n = 3) isolated from 45- to 52-day-old rats were zolpidem sensitive (Fig. 4B). Zolpidem (10–350 nM) was coapplied with 10 μM GABA to obtain a zolpidem concentration-$GABA_A$ receptor current enhancement (response) curve (Fig. 4C). The data

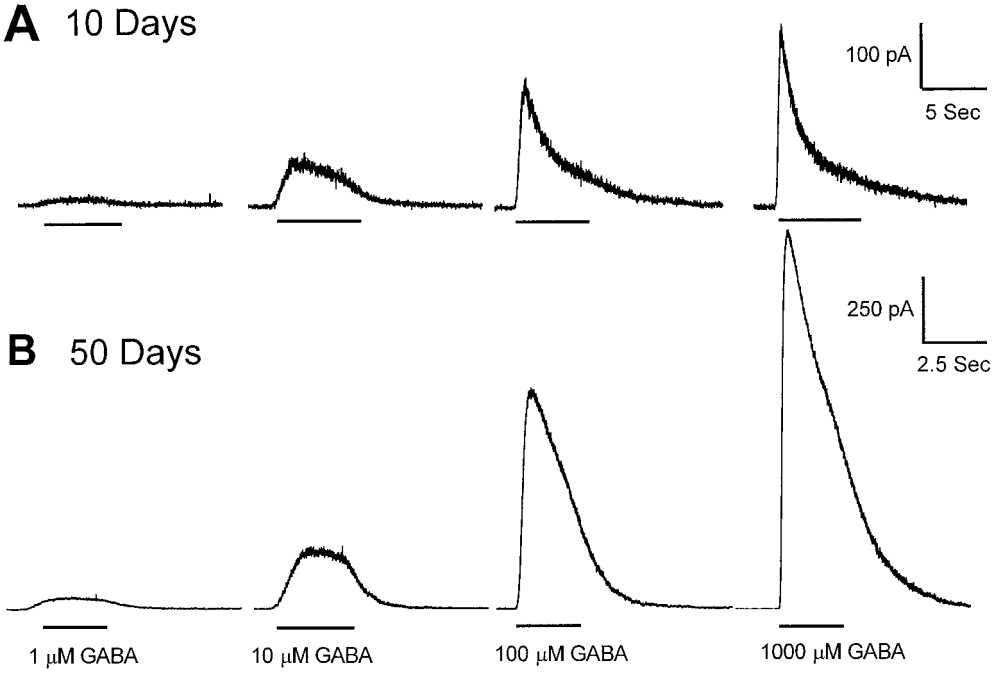

Fig. 1. Larger $GABA_A$ receptor peak currents were recorded from dentate granule cells isolated from 45- to 52-day-old rats than in granule cells isolated from 7- to 14-day-old rats. $GABA_A$ receptor currents were recorded from dentate granule cells isolated from a 10-day-old rat (A) and a 50-day-old rat (B). The duration and concentration of GABA applied are indicated by the bar below each bottom trace.

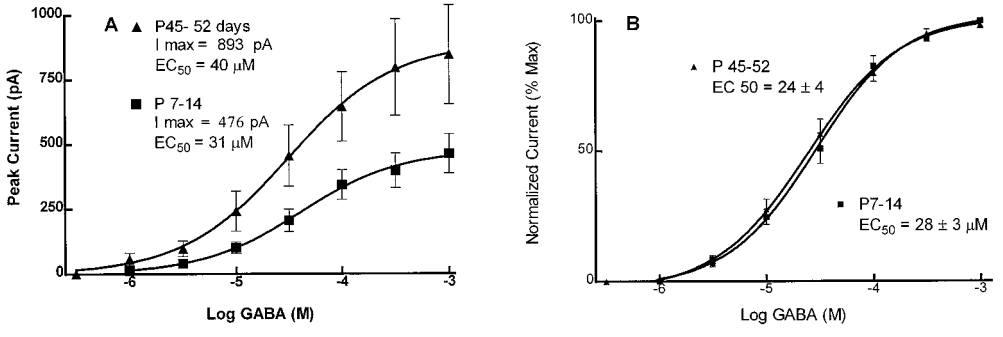

Fig. 2. There was higher efficacy, but no change in potency, of GABA during development. GABA concentration-$GABA_A$ receptor peak current relationships were plotted for groups of hippocampal dentate granule cells acutely isolated from 7- to 14-day-old rats (n = 7, ■) and from 45- to 52-day-old rats (n = 8, ▲). Each point represents the mean of peak currents and error bars show the S.E.M. The line is the best fit of data to a sigmoid function. The maximal current (I_{max}) and EC_{50} values were derived from the equation for the sigmoid function that best fit the data.

were fit to a sigmoidal function with a zolpidem EC_{50} value of 34 ± 17 nM.

Zinc Sensitivity of Granule Cell $GABA_A$ Receptors Declined during Development. The BZ modulation of $GABA_A$ receptor currents in dentate granule cells isolated from 7- to 14-day-old rats suggested the presence of BZ 3 receptors on these cells. Recombinant receptors containing an $\alpha 4$ or $\alpha 6$ subtype, with β and $\gamma 2$ subtypes, have BZ 3 pharmacology (Wieland et al., 1992) with moderate zinc sensitivity [zinc 50% inhibitory concentration (IC_{50}) value of 30–70 μM for inhibition of $GABA_A$ receptor currents] (Knoflach et al., 1996; Saxena and Macdonald, 1996). In contrast, BZ modulation of $GABA_A$ receptor currents in dentate granule cells isolated from 45- to 52-day-old rats suggested the presence of BZ 1 receptors. Recombinant $\alpha 1\beta x\gamma 2$ receptors were minimally zinc sensitive (zinc IC_{50} value of > 100 μM for inhibition of $GABA_A$ receptor currents) (Saxena and Macdonald, 1996).

$GABA_A$ receptor currents from dentate granule cells isolated from 7- to 14-day-old rats had high zinc sensitivity (Fig. 5A). $GABA_A$ receptor currents evoked by 30 μM GABA were inhibited by 100 μM zinc to 46 ± 4% of control (n = 10). In contrast, granule cells isolated from 45- to 52-day-old rats were less sensitive to zinc inhibition (Fig. 5B). $GABA_A$ receptor currents evoked by 30 μM GABA were inhibited by 100 μM zinc to 62± 6% of control ($p < .05$, unpaired t test, n = 7).

This developmental difference in zinc sensitivity of granule-cell $GABA_A$ receptor currents was further characterized by obtaining zinc concentration-response curves for inhibition of peak $GABA_A$ receptor currents (Fig. 5C). Zinc (10–300 μM) was coapplied with 30 μM GABA to cells isolated from 7- to 14-day-old rats (n = 10) and from 45- to 52-day-old rats (n = 7). The data then were fitted to a sigmoidal function. The IC_{50} value for inhibition of $GABA_A$ receptor currents in granule cells isolated from 7- to 14-day-old rats was 40 ± 5 μM whereas that for cells isolated from 45- to 52-day-old rats was 103 ± 19 μM ($p < .05$, unpaired t test). The maximal inhibition was 30 ±7% and 18 ± 11%, respectively ($p > .05$, unpaired t test)

Furosemide Sensitivity of Granule Cell $GABA_A$ Receptor Currents Declined during Development. Dentate granule cells from 7- to 14-day-old rats expressed $GABA_A$ receptors that were relatively insensitive to diazepam and zolpidem but were sensitive to Ro-15–4513 (data not shown), which suggests BZ 3 pharmacology. In contrast, GABA receptors from 45- to 52-day-old rats expressed $GABA_A$ receptors with high diazepam and zolpidem sensitivity, consistent with BZ 1 pharmacology. Recombinant

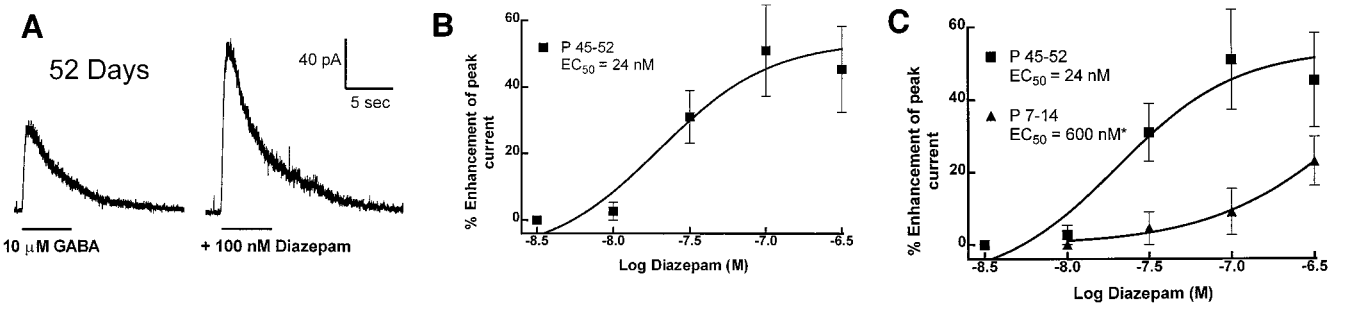

Fig. 3. Sensitivity of $GABA_A$ receptor currents to enhancement by diazepam increased during development. $GABA_A$ receptor currents were recorded from dentate granule cell isolated from a 7-day-old rat (A) and a 52-day-old rat (B). The duration of GABA- and diazepam-application are indicated by the bar below each trace. The drug concentration indicated beneath the bottom trace applies to the top trace also. C, diazepam concentration-$GABA_A$ receptor peak current relationships were plotted for groups of hippocampal dentate granule cells acutely isolated from 7- to 14-day-old (n = 4, ■) and 45- to 52-day-old (n = 5, ▲) rats. Each point represents the mean enhancement of peak current elicited by 10 μM GABA and error bars show the S.E.M. The line is the best fit of data to a sigmoid function. The I_{max} and EC_{50} values were derived from the equation for sigmoid function that best fit the data In granule cells acutely isolated from 7- to 14-day-old rats the data were fit poorly to a sigmoidal function largely because application of high diazepam concentrations (>1 μM) inhibited $GABA_A$ receptor currents and a plateau of maximal enhancement was not obtained. The diazepam EC_{50} for 7- to 14-day-old rats is marked with an asterisk to indicate poor data fit.

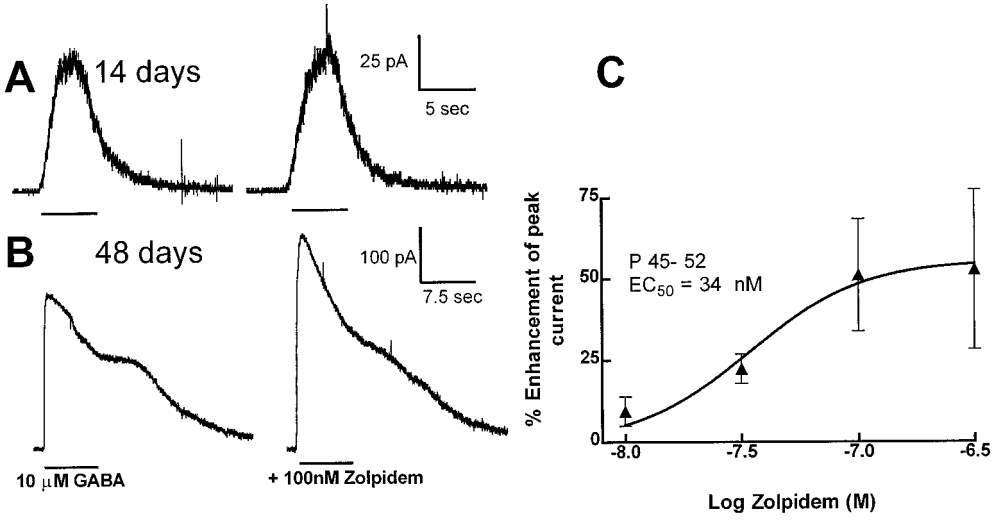

Fig. 4. Sensitivity of $GABA_A$ receptor currents to enhancement by zolpidem increased during development. $GABA_A$ receptor currents were recorded from dentate granule cell isolated from a 14-day-old rat (A) and a 48-day-old rat (B). The durations of GABA and zolpidem application are indicated by the bar below each trace. The drug concentration indicated beneath the bottom trace applies to the top trace also. C, zolpidem concentration-$GABA_A$ receptor peak current relationships were plotted for a group of hippocampal dentate granule cells acutely isolated from 45 to 52 day old rats (n = 3, ▲). Each point represents the mean enhancement of peak currents elicited by 10 μM GABA and error bars show the S.E.M.. The line is the best fit of data to a sigmoid function. The I_{max} and EC_{50} values were derived from the equation for sigmoid function that best fit the data.

$GABA_A$ receptors that contained the $\alpha 1$ subtype and had BZ 1 pharmacology also had low sensitivity to furosemide inhibition of $GABA_A$ receptor currents, whereas $\alpha 4$ or $\alpha 6$ subtype-containing diazepam-insensitive receptors with BZ 3 pharmacology had high sensitivity to furosemide (Wafford et al., 1996).

Furosemide (10 μM to 3 mM) was coapplied with 30 μM GABA to granule cells from 7- to 14-day-old rats ($n = 3$) and 45- to 52-day-old rats ($n = 4$). In granule cells from 7- to 14-day-old rats, peak $GABA_A$ receptor currents were inhibited by concentrations of furosemide ≥ 300 μM (Fig. 6A), but in cells from 45- to 52-day-old rats, 300 μM furosemide did not inhibit the $GABA_A$ receptor currents (Fig. 6B). When fitted to the equation for a sigmoidal function, the IC_{50} value for inhibition of $GABA_A$ receptor currents in granule cells isolated from 7- to 14-day-old rats was 492 ± 79 μM, whereas that for cells isolated from 45- to 52-day-old rats was 1605 ± 199 μM ($p < .05$, unpaired t test, Fig. 6C). The maximal inhibition by furosemide could not be compared because the inhibition curves did not plateau at high furosemide concentrations (Fig. 6C). Higher concentrations of furosemide could not be applied because of its poor solubility.

In our previous study, we did not determine the sensitivity of granule-cell $GABA_A$ receptors to furosemide. To complement the study of developmental changes in furosemide sensitivity of dentate granule-cell $GABA_A$ receptors from rats aged 7 to 14 days and 45 to 52 days, we determined the furosemide sensitivity of dentate granule cells from rats 18 to 30 days old ($n = 6$). In granule cells from 18- to 30-day-old rats, peak $GABA_A$ receptor currents were inhibited by concentrations of furosemide ≥ 300 μM (Fig. 6C). The data could not be fit by a single sigmoidal function. Visual analysis of the data suggested a two-site fit; however, the EC_{50} value for the lower affinity site could not be confidently determined. Visual analysis of the data suggested that furosemide inhibited $GABA_A$ receptor currents at concentrations that were intermediate to those aged 7 to 14 days and 45 to 52 days.

Fraction of Granule Cells Sensitive to Loreclezole Increased during Development. The antiepileptic drug loreclezole has been shown to enhance recombinant $GABA_A$ receptor currents via a specific modulatory site on $GABA_A$ receptor β subunits. The action of loreclezole depended on the β subtype expressed. Isoforms containing $\beta 2$ or $\beta 3$ subtypes had a 300-fold lower EC_{50} value for loreclezole enhancement of $GABA_A$ receptor current than isoforms containing the $\beta 1$ subtype (Wafford et al., 1994). Additionally, at concentrations above 6 μM, loreclezole enhanced the degree and rate of apparent desensitization in a concentration-dependent manner (Donnelly and Macdonald, 1996). This inhibitory effect of loreclezole occurred regardless of subunit composition of the receptor.

The loreclezole sensitivity of dentate granule cells acutely isolated from 7- to 14-day-old rats ($n = 13$) was determined by comparing currents evoked by 10 μM GABA with 10 μM loreclezole to currents evoked by 10 μM GABA alone. In 9 of 13 cells tested, loreclezole (10 μM) reduced $GABA_A$ receptor currents evoked by 10 μM GABA (Fig. 7A). In the remaining four cells tested (30%), 10 μM loreclezole enhanced $GABA_A$ receptor currents evoked by 10 μM GABA. In contrast, in 5 of 7 (70%) granule cells isolated from 45- to 52-day-old rats, 10 μM loreclezole enhanced $GABA_A$ receptor currents evoked by 10 μM GABA (Fig. 7B). In addition to enhancement of $GABA_A$ receptor currents, loreclezole increased the rate of apparent desensitization of $GABA_A$ receptor currents in these neurons. Loreclezole enhancement of $GABA_A$ receptor currents did not correlate with any other pharmacological property of $GABA_A$ receptors. At both ages, loreclezole-sensitive and -insensitive cells had similar BZ and zinc sensitivities.

Discussion

Multiple changes in the pharmacological properties of dentate granule-cell $GABA_A$ receptors occurred during the first 52 days of postnatal development: maximal GABA-evoked current increased with postnatal age; $GABA_A$ receptors changed from type BZ 3 in young rats to type BZ 1 in adult rats; furosemide and zinc inhibited $GABA_A$ receptor currents

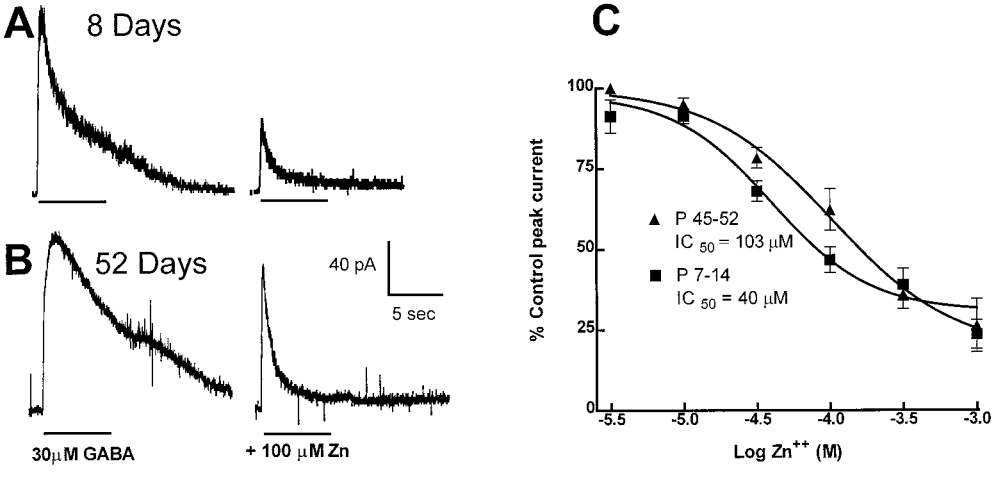

Fig. 5. Sensitivity of $GABA_A$ receptor currents on dentate granule cells to zinc inhibition declined during development. $GABA_A$ receptor currents were recorded from a dentate granule cell isolated from an 8-day-old rat (A) and a 52-day-old rat (B). The durations of GABA and zinc application are indicated by the bar below each trace. The drug concentration indicated beneath the bottom trace applies to the top trace also. C, zinc concentration-$GABA_A$ receptor peak current inhibition relationships were plotted for groups of hippocampal dentate granule cells acutely isolated from 7- to 14-day-old ($n = 10$, ■) and 45- to 52-day-old ($n = 7$, ▲) rats. Each point represents the mean inhibition of peak currents elicited by 30 μM GABA and error bars show the S.E.M. The line was the best fit of data to a sigmoid function. The I_{max} and EC_{50} values were derived from the equation for sigmoid function that best fit the data.

in young rats but not in adult rats; and the fraction of cells expressing loreclezole sensitive $GABA_A$ receptors increased with postnatal age. This transformation was gradual, and granule cells isolated from 28- to 35-day-old rats expressed receptors with pharmacological properties that were intermediate between younger and older rats.

Prolonged Postnatal Development of Dentate Granule Cell $GABA_A$ Receptors. The findings of this study and our previously published study (Kapur and Macdonald, 1996) suggest that the pharmacological properties of dentate granule-cell $GABA_A$ receptors undergo prolonged development lasting up to at least postnatal day 52. There was prolonged postnatal development of $GABA_A$ receptor-mediated, paired-pulse inhibition in the dentate gyrus (Bronzino et al., 1996). The prolonged postnatal maturation of $GABA_A$ receptors was quite similar to the morphological maturation of dentate granule cells (Bayer and Altman, 1974). The granule cell population of the dentate gyrus has the distinctive characteristic of neuronal birth, migration, and death occurring over an extended period of time beginning in gestation and extending to adulthood (Altman and Das, 1965; Gould and Cameron, 1996). The proliferation and migration of granule cells reached its peak in rats in first 2 postnatal weeks and then began to decline (Bayer and Altman, 1974; Schlessinger et al., 1975). The maximum number of immature postnatal granule cells was present at 2 weeks of age and declined to a low level at 2 months of age. Mature granule cells accumulated from the first week; their number reached an asymptotic level at 30 to 70 days.

The findings of this study are of general interest for several reasons. This study directly demonstrated prolonged postnatal development of dentate granule-cell $GABA_A$ receptor properties, which is a novel finding. Several current models of central nervous system GABAergic neurotransmission are based on the study of inhibitory synapses present on dentate granule cells (Edwards et al., 1990; Mody et al., 1994). The GABA-mediated IPSCs in dentate granule cells change dramatically during development (Hollrigel and Soltesz, 1997). The current study suggests that these changes are caused, at least in part, by altered $GABA_A$ receptor properties. Additionally, GABA may play different roles in the dentate gyrus during development; expression of pharmacologically distinct $GABA_A$ receptors at each stage of development may be one mechanism by which this is accomplished. Finally, these findings may partly explain differences between epilepsy in childhood and adults. Childhood epilepsy differs from adult epilepsy in its clinical and EEG manifestations, response to anticonvulsant drugs, etiological factors, and outcome. Altered $GABA_A$ receptor-mediated inhibition of the dentate gyrus is the basis of several hypotheses of pathogenesis of epilepsy (Mody, 1998) and many drugs currently used to treat epilepsy exert their anticonvulsant effect by acting on $GABA_A$ receptors (Macdonald and Kelly, 1995). Our findings that $GABA_A$ receptors expressed on dentate granule cells of young rats are distinct from those on adult rats may partly explain differences between childhood and adult epilepsy.

Postnatal Development of $GABA_A$ Receptor Currents. The maximal current elicited by GABA increased significantly from day 14 to day 28 and then remained stable at age 52 days (Table 1). The increase in the magnitude of whole cell $GABA_A$ receptor currents observed during postnatal development could have been caused by an increased receptor density present at each synapse, an increase in the density of extrasynaptic receptors, an increase in the number of GABAergic synapses on each dentate granule cell, or a combination of these factors. An increase in the density of synaptic $GABA_A$ receptors seemed unlikely, because the peak amplitude of miniature IPSCs did not change during development (Hollrigel and Soltesz, 1997) and the miniature IPSC peak current was determined by the number of postsynaptic $GABA_A$ receptors (Edwards et al., 1989; Mody et al., 1994). An increase in the size of granule cells with an associated proportional increase in synaptic and extrasynaptic $GABA_A$ receptors could explain the larger whole cell

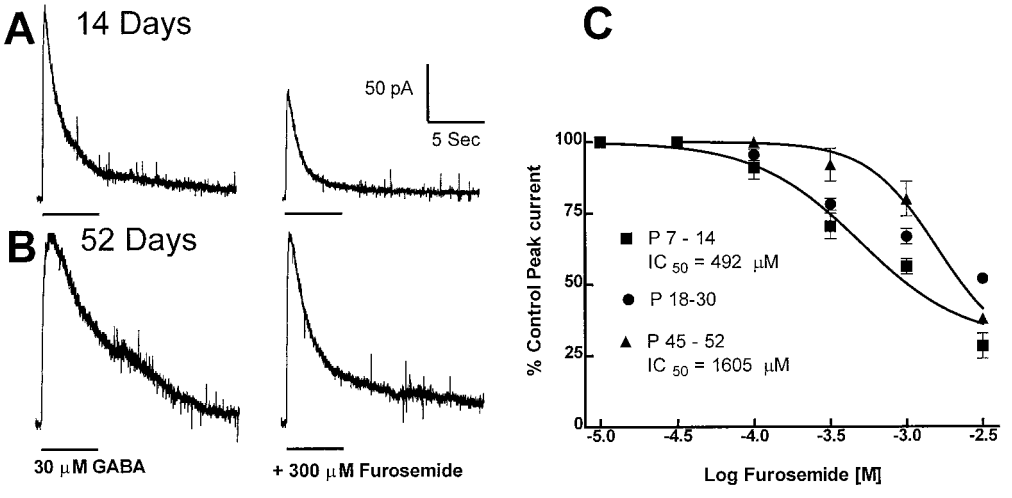

Fig. 6. Sensitivity of $GABA_A$ receptor currents on dentate granule cells to furosemide inhibition declined during development. $GABA_A$ receptor currents were recorded from a dentate granule cell isolated from a 14-day-old rat (A) and a 52-day-old rat (B). The durations of GABA and furosemide application are indicated by the bar below each trace; the drug concentration indicated beneath the bottom trace applies to the top trace also. C, furosemide concentration-$GABA_A$ receptor peak current inhibition relationships were plotted for groups of hippocampal dentate granule cells acutely isolated from 7- to 14-day-old (n = 3, ■), 18- to 30-day-old (n = 6, ●), and 45- to 52-day-old (n = 4, ▲) rats. Each point represents the mean inhibition of peak currents elicited by 30 μM GABA and error bars show the S.E.M.. The line was the best fit of data to a sigmoid function. The maximal inhibition I_{max} and EC_{50} values were derived from the equation for sigmoid function that best fit the data. The data from neurons isolated from 18- to 30-day-old rats were not fit to a sigmoidal function.

$GABA_A$ receptor currents in cells from older rats. Histological studies demonstrated that immature granule cells were small, whereas mature cells could be small or large, and the number of mature granule cells continued to increase up to postnatal day 70 (Bayer and Altman, 1974). This could result in increased GABAergic synapses on mature granule cells and increased extrasynaptic $GABA_A$ receptors. However, the current study did not systematically evaluate cell dentate granule cell volume at various ages by capacitance measurement. Thus the increase in $GABA_A$ receptor currents could have resulted from a combination of these factors.

Postnatal Development of BZ, Zinc, and Furosemide Modulation of Dentate Granule Cell $GABA_A$ Receptor Currents. Dentate granule cells from 7- to 14-day-old rats expressed $GABA_A$ receptor currents that were poorly enhanced by diazepam and zolpidem, whereas $GABA_A$ receptor currents from 45- to 52-day-old rats were enhanced with high affinity by diazepam and zolpidem (Table 1). These data suggested that BZ 3 receptors were expressed early in postnatal development and were transformed to BZ 1 receptors by 45 to 52 days of age. $GABA_A$ receptors present on cells from 28- to 35-day-old rats (Table 1) had intermediate BZ sensitivity and could not be classified as BZ 1, BZ 2, or BZ 3 receptors (for details, see Kapur and Macdonald, 1996). Data from the previous study combined with those reported here suggest that transformation of granule-cell $GABA_A$ receptors from BZ 3 to BZ 1 receptors is a gradual process with a clear intermediate state.

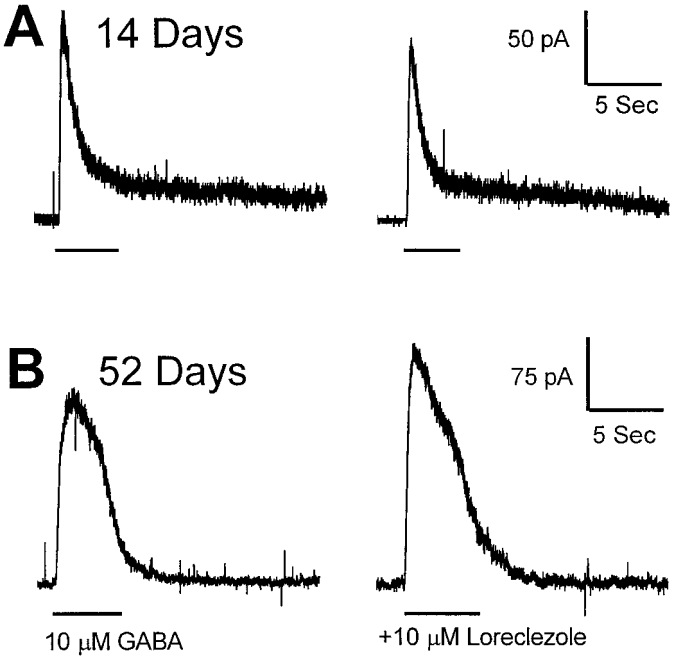

Fig. 7. Loreclezole effects on granule-cell $GABA_A$ receptor currents changed during development. $GABA_A$ receptor currents were recorded from dentate granule cells isolated from a 14-day-old rat (A) and a 52-day-old rat (B). The durations of GABA and loreclezole application are indicated by the bar below each trace. The drug concentration indicated beneath the bottom trace applies to the top trace also. Note inhibition of peak current by loreclezole in A and enhancement of the apparent current desensitization in trace B (see text for details).

The zinc sensitivities of $GABA_A$ receptor currents in granule cells isolated from young 7- to 14-day-old rats and immature 28- to 35-day-old rats were similar (Table 1). The developmental diminution of dentate granule-cell $GABA_A$ receptor current sensitivity to zinc occurred late, between postnatal days 35 and 45. This finding further suggests that postnatal development of $GABA_A$ receptor is an extended process that lasts 45 to 52 days.

The postnatal development of furosemide sensitivity of dentate granule-cell $GABA_A$ receptor currents also showed a gradual shift from high sensitivity in 7- to 14-day-old rats to a lower sensitivity in rats aged 45 to 52 days. Similar to the development of BZ sensitivity, the cells at the intermediate ages expressed receptors with intermediate properties. This further supports the notion that there was gradual transformation of $GABA_A$ receptors from BZ 3 at young ages to BZ 1 receptors by 45 to 52 days of age (Table 1).

Possible Molecular Bases for Developmental Changes in BZ, Zinc, and Furosemide Modulation of Dentate Granule Cell $GABA_A$ Receptor Currents. The most likely explanation for changes in the pharmacological properties of dentate granule-cell $GABA_A$ receptors during development was that different receptor isoforms were expressed at 7 to 14, 28 to 35, and 45 to 52 days of age. The pharmacological properties of $GABA_A$ receptors in granule cells from 7- to 14-day-old rats were similar to those of recombinant receptors containing $\alpha 4$, $\gamma 2$, and $\beta 1$ or $\beta 3$ subtypes. The presence of an $\alpha 4$ or $\alpha 6$ subtype along with a $\gamma 2$ subtype would explain the low affinity for diazepam, moderate affinity for zinc, and relatively high sensitivity to furo-

TABLE 1

A comparison of pharmacological properties of $GABA_A$ receptors present on dentate granule cells acutely isolated from 7- to 14-day-old, 28- to 35-day-old, and 45- to 52-day-old rats

Drug	Age		
	7–14 Days	28–35 Days[a]	45–52 Days
GABA (I_{max})[b,c,d]	476 ± 65 pA	842 ± 54 pA	893 ± 160 pA
GABA (EC_{50})	40 ± 8 μM	46 ± 10 μM	31 ± 10 μM
Diazepam (EC_{50})[e]	648 nM	158 ± 13 nM	24 ± 7 nM
Zolpidem (EC_{50})		75 ± 13 nM	34 ± 17 nM
Furosemide (IC_{50})[d]	492 ± 79 μM	Biphasic	1605 ± 199 mM
Zinc (IC_{50})[a,d,e]	40 ± 5 μM	28 ± 11 μM	103 ± 19 μM
Loreclezole (% sensitive cells)[f]	30	50	70

[a] These data were from a previous publication by the authors using the same techniques used in the current experiments.
[b] $P < .05$, one-way ANOVA.
[c] $P < .05$, Newman-Keuls multiple-comparison test between columns 7–14 days and 28–35 days.
[d] $P < .05$ Newman-Keuls multiple-comparison test between columns 7–14 days and 45–52 days. Grouped t test was used when only two cells of data were compared, for drugs diazepam, zolpidem, and furosemide.
[e] $P < .05$ Newman-Keuls multiple-comparison test between columns 28–35 days and 45–52 days.
[f] $P < .05$, χ^2 test comparison between columns 7–14 days and 45–52 days.

semide. $GABA_A$ receptors in 45- to 52-day-old rats had high diazepam and zolpidem sensitivities but were relatively insensitive to zinc and furosemide. Recombinant receptors containing α1, γ2, and βx subtypes also have these properties.

This interpretation was supported by immunohistochemical studies demonstrating age-dependent changes in expression of α1 and α2 subtype immunoreactivity on dentate granule cells (Fritschy et al., 1994). α1 Subtype immunoreactivity was low in dentate granule cells at birth and increased by 20 days of age. α2 Subtype immunoreactivity was present at birth and continued unchanged to 20 days of age. Coexpression of α1 and α2 subtype immunoreactivity on same neurons was directly demonstrated by confocal laser microscopy in several regions. Coexpression of two α subtypes on dentate granule cells at 28 to 35 days of age could explain their intermediate BZ and furosemide sensitivity. The developmental changes in α subtype mRNA also followed the pattern of low α1 mRNA at birth and high α2 mRNA expression from birth to adulthood (Laurie et al., 1992). The immunocytochemical distribution of 13 different $GABA_A$ receptor subunits in the hippocampus of adult rats was described recently (Sperk et al., 1997). High concentrations of α1, α2, α4, β3, γ2, and δ but no α6 immunoreactivities were observed within the molecular layer of the dentate gyrus. The pharmacological properties of $GABA_A$ receptors on 45- to 52-day-old rats were consistent with the expression of at least three of these subunits, α1, β3, γ2, and δ.

Other possible explanations for the developmental changes in the pharmacological properties of granule-cell $GABA_A$ receptors included the absence of a γ2 subtype in receptors on granule cells from young rats or post-translational modification of receptors. However, absence of the γ2 subtype would be inconsistent with the presence of moderate zinc sensitivity and enhancement by high concentrations of diazepam. Also, modification of $GABA_A$ receptor pharmacological properties by post-translational modification has not been demonstrated.

Development of Loreclezole Sensitivity. The action of loreclezole on recombinant $GABA_A$ receptors depends on the β subtype expressed. Isoforms containing β2 or β3 subtypes had a 300-fold lower EC_{50} value for loreclezole enhancement of $GABA_A$ receptor current than isoforms containing the β1 subtype (Wafford et al., 1994). High loreclezole sensitivity depends on the presence of a β2 or β3 subtype without a β1 subtype (Fisher and Macdonald, 1997). This suggests that 30% of granule cells in 7- to 14-day-old rats, 50% of granule cells in 28- to 35-day-old rats, and 70% of granule cells in 45- to 52-day-old rats expressed $GABA_A$ receptor with only β2 and/or β3 subtypes. The continued presence of granule cells expressing $GABA_A$ receptors not enhanced by loreclezole at 45 to 52 days of age may reflect continued presence of immature granule cells to this age (Bayer and Altman, 1974). In addition to enhancement of peak $GABA_A$ receptor currents, loreclezole was demonstrated to enhance the degree and rate of apparent desensitization in a concentration-dependent manner (Donnelly and Macdonald, 1996). This inhibitory effect of loreclezole occurred regardless of subunit composition of the receptor and was apparent in cells isolated from all three age groups.

$GABA_A$ Receptor Development in Other Regions of the Brain. During development, changes in the properties of $GABA_A$ receptors present in many different regions of the brain have been described. The properties and subunit composition of $GABA_A$ receptors on cultured cerebellar granule cells changed during development (Mathews et al., 1994). Cerebellar granule cells expressed BZ-sensitive α1-subtype-containing $GABA_A$ receptors after 5 to 7 days in culture, but mature granule cells, 21 to 25 days in culture, expressed α6-containing BZ-insensitive $GABA_A$ receptors. In hippocampal CA3 neurons in slices from young animals before 15 days of age, zolpidem enhanced IPSPs poorly and BZ action was mediated through BZ 2 receptors, whereas in slices from adult animals, BZ 1 receptors were present (Rovira and Ben-Ari, 1993). $GABA_A$ receptors present on rat thalamic reticular neurons (Gibbs, III et al., 1996) and principal neurons (Oh et al., 1995) also undergo developmental regulation with changes in maximal current and BZ regulation.

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