Tonic and Synaptically Evoked Presynaptic Inhibition of Sensory Input to the Rat Olfactory Bulb Via GABA_B Heteroreceptors

Vassiliki Aroniadou-Anderjaska, Fu-Ming Zhou, Catherine A. Priest, Matthew Ennis, and Michael T. Shipley

Department of Anatomy and Neurobiology and Program in Neuroscience, University of Maryland School of Medicine, Baltimore, Maryland 21201

Received 3 February 2000; accepted in final form 24 May 2000

INTRODUCTION

Olfactory sensory input is transduced by olfactory receptor neurons (ORNs) in the nasal epithelium and relayed to the glomeruli of the main olfactory bulb (OB) via the olfactory nerve (ON). Mammalian ORNs are thought to express a single odorant receptor (Buck and Axel 1991; Chess et al. 1994; Kishimoto et al. 1994; Zhao et al. 1998). A cohort of ORNs expressing the same odorant receptor projects to two (or a few) topographically fixed glomeruli in the olfactory bulb (Mombaerts et al. 1996; Ressler et al. 1994; Vassar et al. 1994). Odorant receptors do not appear to be specific to an odor but rather to molecular features that may be present in many odorants (Johnson et al. 1998). Thus a given odor activates several cohorts of ORNs (those that respond to the molecular features of the odorant) and evokes a specific pattern of glomerular activity (Buck 1996; Friedrich and Korsching 1997; Johnson and Leon 1996; Johnson et al. 1998; Laurent 1996). A key issue in olfaction is how the brain computes odors from patterns of glomerular activity. While the glomeruli are the initial stage in this computation (Shepherd 1994), little is known about the intraglomerular synaptic mechanisms that regulate glomerular activity.

In the glomeruli, ON terminals form excitatory, glutamatergic synapses with the apical dendrites of mitral/tufted (M/T) cells and JG cells, with no evidence for postsynaptic effects. Baclofen (0.5–1 μM) also reversed paired-pulse depression (PPD) of mitral/tufted cell responses to paired-pulse facilitation (PPF), and reduced depression of JG cell excitatory postsynaptic currents (EPSCs) during repetitive ON stimulation. These results suggest that baclofen reduced the probability of glutamate release from ON terminals. The GABA_B antagonists CG35348 or CGP55845A increased mitral/tufted cell responses evoked by single-pulse ON stimulation, suggesting that glutamate release from ON terminals is tonically suppressed via GABA_B receptors. The same antagonists reduced PPD of ON-evoked mitral/tufted cell responses at interstimulus intervals 50–400 ms. This finding suggests that a single ON impulse evokes sufficient GABA release, presumably from JG cells, to activate GABA_B receptors on ON terminals. Thus GABA_B heteroreceptors on ON terminals are activated by ambient levels of extrasynaptic GABA, and by ON input to the OB. The time course of ON-evoked, GABA_B presynaptic inhibition suggests that neurotransmission to M/T cells and JG cells will be significantly suppressed when ON impulses arrive in glomeruli at 2.5–20 Hz. GABA_B receptor–mediated presynaptic inhibition of sensory input to the OB may play an important role in shaping the activation pattern of the OB glomeruli during olfactory coding.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
receptors on ON terminals (Bonino et al. 1999) supports the contention that the effects of baclofen were mediated, at least in part, presynaptically (Keller et al. 1998; Nickell et al. 1994). The purpose of the present study was to investigate the role that GABA_β receptors on ON terminals play in the function of the glomeruli.

M E T H O D S

Slices of the main olfactory bulb (OB) were prepared from Wistar rats, 17–22 days old. Details of the slice preparation have been described previously (Aroniadou-Anderjaska et al. 1997). Briefly, the rats were anesthetized with chloral hydrate (400 mg/kg body wt) followed by whole-body immersion in ice-cold water. The brain with the two bulbs was removed and glued to the stage of a Vibroslicer. Some of the experiments required the lateral olfactory tract (LOT) to be preserved in the slices (Fig. 1A). To accomplish this, the brain was positioned so that the bulbs were in approximately the same horizontal plane with the most ventral part of the forebrain. Slices, 450–500 μm thick, were cut and transferred to an interface chamber, maintained at 33°C, for field potential and intracellular recordings, while whole cell recordings were performed in submerged slices at room temperature. The slices were perfused with artificial cerebrospinal fluid (in mM: 124 NaCl, 26 NaHCO3, 1.2 NaH2PO4, 3 KCl, 1.3 MgSO4, 2.5 CaCl2, and 10 glucose) at a rate of 1 ml/min in the interface chamber, and 3 ml/min in the submerged conditions. In some experiments, medium with nominally zero concentration of Mg^2+ was used; this medium did not include MgSO4.

Experiments were initiated 1–2 h after the slices were placed in the chamber. Field potentials were recorded in the GL and, in some experiments, in the granule cell layer (GCL; see basic OB circuitry in Fig. 1), using glass pipettes (0.5–2 MΩ) filled with 2 N NaCl. They were filtered (3 kHz low-pass), and digitized on-line at 15 kHz. Extracellular stimulation was applied to the ON or the LOT (Fig. 1A) using a dipolar stainless steel electrode (50 μm diam). Stimulus pulses were 100 μs duration and were applied at 0.05 Hz. Unless indicated otherwise in the results, stimulus intensity was adjusted to evoke a field potential of 1.5–2.5 mV in the GL by ON stimulation (10–60 μA), or in the GCL by LOT stimulation (80–150 μA). This amplitude range was 40–70% of the maximum peak amplitude of ON-evoked GL field potentials, and 60–80% of the maximum amplitude of LOT-evoked GCL field potentials.

One set of experiments required recordings of laminar field potential profiles, which were analyzed with the current source density (CSD) method as described previously (Aroniadou-Anderjaska et al. 1999a). Briefly, the approximation formula for one-dimensional CSD (Freeman and Nicholson 1975) was used. The validity of one-dimensional CSD in the main OB, along the axis perpendicular to the laminae, has been shown (Aroniadou-Anderjaska et al. 1999a). Spatial resolution and differentiation grid were 100 and 200 μm, respectively. Conductivity gradients across laminae have no significant influence on the general features of the laminar CSD distribution (Martinez 1982), and therefore they were considered negligible.

Conventional methods were used for intracellular recordings from mitral cells, using the Axoclamp-2A amplifier (Axon Instruments) in the bridge mode. Glass pipettes (50–90 MΩ) were filled with potassium acetate (4 M). For whole cell recordings from juxtaglomerular cells, patch pipettes were filled with (in mM) 135 KCl, 10 HEPEs, 2 Mg-ATP, 0.2 Na-GTP, and 0.5 EGTA; pH and osmolarity were adjusted to 7.3 and 280 mOsm, respectively. Electrical signals were recorded using an Axopatch-200B amplifier (Axon Instruments) with the low-pass filter set at 5 kHz. Juxtaglomerular cells were visualized with an Olympus BX50WI upright microscope equipped with a ×60 water immersion lens and DIC optics. The recorded cells were probably the periglomerular type (GABAergic juxtaglomerular cells) because they had small soma size and high-input resistance. All data acquisition and analysis, including the CSD analysis, were performed with the pClamp software (Axon Instruments). In the results, group data are presented as means ± SE.

The following drugs were used: R(+) baclofen hydrochloride (referred to as baclofen), a selective GABA_β receptor agonist (Research Biochemicals International); CGP-35348 (Olpe et al. 1990) or CGP-55845A (Frostl et al. 1992), specific, competitive GABA_β receptor antagonists (gift from NOVARTIS Pharma); 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), a kainate/α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist (Research Biochemicals International); d-2-amino-5-phosphonovalerate (APV), an N-methyl-D-aspartate (NMDA) receptor antagonist (Research Biochemicals International); and bicuculline methchloride, a GABA_A receptor antagonist (Research Biochemicals International). To prepare stock solutions, all drugs were dissolved in dH2O, except for CNQX, which was dissolved in DMSO (final concentration of DMSO in the slice medium was 0.01%, vol/vol). All drugs were delivered by bath application.
After addition of baclofen (5 \( \mu \)M), the effects of GABA \( \beta \) receptor activation by baclofen on the layers, baclofen (5 \( \mu \)M) also reduced the glomerular field EPSP. Traces are averages of 5–10 sweeps.

**RESULTS**

**Pharmacological activation of GABA\( \beta \) receptors**

**EFFECTS OF BACLOFEN ON ON-EVOKED RESPONSES OF M/T CELLS.** In the first series of experiments, our aim was to obtain solid physiological evidence for the presence of functional GABA\( \beta \) receptors on ON terminals. First, we examined the effects of GABA\( \beta \) receptor activation by baclofen on the ON-evoked responses of M/T cells. Single pulses to the ON evoke a two-component field excitatory postsynaptic potential (EPSP) in the GL (Fig. 2A). This field potential reflects predominantly synaptic currents in the apical dendrites of M/T cells and is mediated by glutamate receptors (Aroniadou-Anderjaska et al. 1997, 1999a). The fast component (N1) is mediated by kainate/AMPA receptors, whereas most of the slow component (N2) depends on NMDA receptor activation. Bath application of 5 \( \mu \)M baclofen reduced both the N1 and N2 components of the field EPSP (Fig. 2B). The peak amplitude of N1 was reduced by 79.6 \( \pm \) 5.6% (mean \( \pm \) SE, \( n = 10 \)). These effects were reversed after washing-out baclofen.

In slices where the GL was surgically isolated from the deeper layers, baclofen (5 \( \mu \)M) also reduced the ON-evoked field EPSP. Traces are averages of 5–10 sweeps.

**FIG. 2.** Baclofen suppresses ON-evoked field excitatory postsynaptic potentials (EPSPs) of mitral/tufted (M/T) cells. A: the field EPSP generated by the glomerular dendritic tufts of M/T cells in response to ON stimulation consists of a fast component (N1), which is blocked by 6-cyano-7-nitroquininaline-2,3-dione (CNQX; 10 \( \mu \)M), and a slow component (N2), most of which is blocked by \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (APV; 50 \( \mu \)M). B: baclofen (5 \( \mu \)M) reversibly suppressed the ON-evoked glomerular field EPSPs. C: in slices where the GL was surgically isolated from the deeper layers, baclofen (5 \( \mu \)M) also reduced the ON-evoked field EPSP. Traces are averages of 5–10 sweeps.

**FIG. 3.** ON-evoked responses recorded intracellularly from mitral cells are completely suppressed by baclofen. The cell shown (resting membrane potential \(-64 \text{ mV}\) ) generated a prolonged EPSP in response to 20 \( \mu \)A stimulation. The GL field potential (FP), recorded simultaneously, is shown above each trace of the intracellular response. When the stimulus intensity was increased to 40 \( \mu \)A, the EPSP was preceded by a fast prepotential, which gave rise to an action potential following further increase of stimulation intensity (70 \( \mu \)A). Baclofen (5 \( \mu \)M) significantly reduced or blocked the field and cell responses even at the higher stimulus intensities. The effects of baclofen were largely reversed by CGP-35348 (500 \( \mu \)M). Traces are averages of 5 sweeps.

**SITE OF BACLOFEN ACTION.** The dramatic effect of baclofen on ON-evoked responses of M/T cells suggested that baclofen may act on the ON terminals and inhibit glutamate release. The apparently minor postsynaptic effects on mitral cells were consistent with this possibility; however, if GABA\( \beta \) receptors on mitral cells were present exclusively on the apical dendritic tufts, postsynaptic effects of baclofen may be difficult to detect with somatic recordings.

To determine whether baclofen acted presynaptically, first we examined how baclofen affects responses to paired-pulse stimulation of the ON. When two identical stimuli are delivered at various interstimulus intervals (ISI), the first pulse may produce depression (paired-pulse depression, PPD) or facilitation (PPF) of the response to the second pulse. An important factor determining whether PPD or PPF is produced is the probability of transmitter release in response to the first (conditioning) pulse (Debanne et al. 1996; Manabe et al. 1993; Thomson et al. 1993). The present paired-pulse experiments were carried out in the presence of APV (50–100 \( \mu \)M) to suppress the long-lasting N2 component of the field potential, and thus allow a more accurate measurement of changes in the kainate/AMPA component. Prior to addition of baclofen, paired-pulse stimulation of the ON produced depression of the second (test) response at ISIs starting from 10 ms. The longest ISI at which PPD was still present ranged from 400 to 700 ms (542.8 \( \pm \) 43.5 ms, \( n = 8 \)). Baclofen (0.5–1 \( \mu \)M) reduced disproportionately the conditioning versus test responses, resulting in the reversal of PPD to PPF (Fig. 4A, 4C). The effects of baclofen were significant at all ISIs tested, i.e., 100, 200, and 300 ms (\( P < 0.05 \), paired \( t \)-test). Increasing the stimulus intensity to bring the conditioning response magnitude closer to the control, still produced PPF (Fig. 4Ac). The effects of baclofen were reversible (Fig. 4Ad). These results suggest that baclofen reduces the probability of glutamate release.
baclofen can block ON input to M/T cells, by a presynaptic action alone.

A final test to determine whether baclofen can completely suppress synaptic transmission from the ON, without any postsynaptic effects, was to investigate the effects of baclofen on ON-evoked synaptic currents of JG cells. Any potential postsynaptic effects of baclofen on JG cells should be measurable because, due to their small size, JG cells can be voltage clamped effectively. AMPA/kainate excitatory postsynaptic currents (EPSCs) were isolated by adding APV (50 μM) and bicuculline (10 μM) to the medium. Addition of baclofen (2 μM) completely suppressed the ON-evoked EPSCs (Fig. 6A, n = 4); this effect was not associated with any measurable change in input resistance (2.3 ± 0.2 GΩ, n = 4) or holding current (−70 mV). Thus baclofen can completely suppress synaptic transmission from the ON to JG cells by a presynaptic action alone. Lower concentrations of baclofen (0.5–1 μM) that reduced but did not completely suppress the ON-evoked synaptic currents, reduced the depression of these currents during repetitive (5 Hz) stimulation (n = 4, Fig. 6B). This is consistent with the interpretation that baclofen reduces the probability of glutamate release from the ON terminals.

**Activation of GABAB receptors by endogenous GABA**

To investigate whether GABAB receptors on ON terminals play a role in the function of the OB, we next examined whether these receptors are activated by endogenous GABA. The origin of endogenous GABA in the glomeruli is a population of JG cells, the GABAergic periglomerular (PG) cells (Gall et al. 1987; Kosaka et al. 1985; Mugnaini et al. 1984; Ribak et al. 1977a). PG cells receive excitatory input from the ON (present data and Bardoni et al. 1996; Heyward et al. 1997; Keller et al. 1998) and from the dendrites of M/T cells (Bardoni et al. 1996). Ultrastructural studies have revealed symmetrical synapses from PG cells to the dendrites of M/T cells and other PG cells, but no evidence has been found for synapses from PG cells onto ON terminals (Hinds 1970; Pinching and Powell 1971b; Ribak et al. 1977b).

To determine whether, despite the lack of anatomical synapses, GABA released from PG cells gains access to GABAergic receptors on ON terminals, we investigated the effects of the GABAB antagonists, CGP-35348 (0.5–1 mM) and CGP-55845A (10 μM), on glomerular field EPSPs evoked by paired-pulse ON stimulation. The GABAB antagonists increased both the conditioning and the test responses. The effect on the test responses was significantly greater, resulting in reduction of PPD, or reversal of PPD to PPF. An example is shown in Fig. 7A, and group data from 10 slices where CGP-35348 was used are shown in Fig. 7B. The reduction in PPD by CGP-35348 was significant at interstimulus intervals from 50 to 400 ms (P < 0.02, paired t-test). These results suggest that GABA released from PG cells in response to the conditioning pulse of the ON activates GABAB receptors on ON terminals and thus reduces the M/T cell response to the test pulse.

The responses to the conditioning pulses, and responses to single-pulse ON stimulation, were also increased by the GABAB antagonists (Fig. 7, A and C). The peak amplitude of synaptic responses of M/T cell dendrites, without causing any significant change in the response of the same dendrites to another glutamatergic input. These results indicate that baclofen can block ON input to M/T cells, by a presynaptic action alone.

**Activation of GABAB receptors by endogenous GABA**

To investigate whether GABAB receptors on ON terminals play a role in the function of the OB, we next examined whether these receptors are activated by endogenous GABA. The origin of endogenous GABA in the glomeruli is a population of JG cells, the GABAergic periglomerular (PG) cells (Gall et al. 1987; Kosaka et al. 1985; Mugnaini et al. 1984; Ribak et al. 1977a). PG cells receive excitatory input from the ON (present data and Bardoni et al. 1996; Heyward et al. 1997; Keller et al. 1998) and from the dendrites of M/T cells (Bardoni et al. 1996). Ultrastructural studies have revealed symmetrical synapses from PG cells to the dendrites of M/T cells and other PG cells, but no evidence has been found for synapses from PG cells onto ON terminals (Hinds 1970; Pinching and Powell 1971b; Ribak et al. 1977b).

To determine whether, despite the lack of anatomical synapses, GABA released from PG cells gains access to GABAergic receptors on ON terminals, we investigated the effects of the GABAB antagonists, CGP-35348 (0.5–1 mM) and CGP-55845A (10 μM), on glomerular field EPSPs evoked by paired-pulse ON stimulation. The GABAB antagonists increased both the conditioning and the test responses. The effect on the test responses was significantly greater, resulting in reduction of PPD, or reversal of PPD to PPF. An example is shown in Fig. 7A, and group data from 10 slices where CGP-35348 was used are shown in Fig. 7B. The reduction in PPD by CGP-35348 was significant at interstimulus intervals from 50 to 400 ms (P < 0.02, paired t-test). These results suggest that GABA released from PG cells in response to the conditioning pulse of the ON activates GABAB receptors on ON terminals and thus reduces the M/T cell response to the test pulse.

The responses to the conditioning pulses, and responses to single-pulse ON stimulation, were also increased by the GABAB antagonists (Fig. 7, A and C). The peak amplitude of synaptic responses of M/T cell dendrites, without causing any significant change in the response of the same dendrites to another glutamatergic input. These results indicate that baclofen can block ON input to M/T cells, by a presynaptic action alone.
the effects of the GABA B antagonists on the GL field EPSP (Aroniadou-Anderjaska et al. 1997, 1999a). Thus if there were EPSP, increasing its amplitude and broadening its time course extent the fast, kainate/AMPA component of the GL field EPSP evoked by ON stimulation influences to some tive field potential in the GCL (Rall and Shepherd 1968). The external plexiform layer (EPL) and a corresponding posi-

dendrites of granule cells producing a negative field EPSP in

or CGP-35348 M/T to granule cell transmission is not affected by baclofen or CGP-35348

N1 increased by 30%, from 2.0 ± 0.13 mV, in control medium, to 2.6 ± 0.18 mV, after application of either GABA\textsubscript{B} antagonist (\(n = 19, P < 0.0004\), paired \(t\)-test). Figure 7C shows the time course of the effect of CGP-55845A on conditioning and test responses evoked at an interstimulus interval of 100 ms (group data from 6 slices). The latency to peak amplitude of N1 ranges from 9 to 12 ms, whereas ON-evoked activation of GABA\textsubscript{B} receptors on ON terminals takes effect after 30 ms post-ON stimulation (Fig. 7B). Therefore the increase of the conditioning responses by the GABA\textsubscript{B} antagonists cannot be due to the same mechanism as the test responses, i.e., blockade of polysynaptically mediated activation of GABA\textsubscript{B} receptors; rather, this increase suggests that the GABA\textsubscript{B} antagonists blocked tonic activation of GABA\textsubscript{B} receptors. Thus taken together, these results show that GABA\textsubscript{B} receptors on ON terminals are tonically activated by ambient concentrations of extracellular GABA, and are further activated by GABA release from PG cells in response to a single stimulus pulse applied to the ON.

M/T to granule cell transmission is not affected by baclofen or CGP-35348

The lateral dendrites of M/T cells release glutamate onto the dendrites of granule cells producing a negative field EPSP in the external plexiform layer (EPL) and a corresponding positive field potential in the GCL (Rall and Shepherd 1968). The EPL field EPSP evoked by ON stimulation influences to some extent the fast, kainate/AMPA component of the GL field EPSP, increasing its amplitude and broadening its time course (Aroniadou-Anderjaska et al. 1997, 1999a). Thus if there were GABA\textsubscript{B} receptors on the lateral dendrites of M/T cells, then the effects of the GABA\textsubscript{B} antagonists on the GL field EPSP (Fig. 7) could, in part, be due to a change in the EPL field EPSP. To investigate this possibility we delivered stimulus pulses alternately to the LOT and to the ON, while recording granule cell field responses in the GCL. Baclofen (up to 50 \(\mu M\)) had no effect on the LOT-evoked field potential, although it completely suppressed the ON-evoked GCL field potential (\(n = 3\), Fig. 8A). Similarly, CGP-35348 had no effect on PPD of the LOT-evoked, GCL field potential, although it reduced PPD of the ON-evoked GCL field potential (ISIs 50, 100, 150, and 200 ms; \(n = 4\), Fig. 8B). Thus baclofen and CGP-35348 do not affect transmission from M/T to granule cells; the effects of these drugs on the ON-evoked GCL field potential were due to activation of GABA\textsubscript{B} receptors on the ON terminals. These results allow us to conclude that the effects of the GABA\textsubscript{B} antagonists on the ON-evoked, GL field EPSP (Fig. 7) are solely due to an action in the GL.

**DISCUSSION**

In the present study, it was found that 1) baclofen suppresses synaptic transmission from the ON to M/T cells and JG cells by reducing glutamate release from the ON axon terminals, and 2) GABA\textsubscript{B} receptors on ON terminals are activated by endoge-

**Evidence for functional GABA\textsubscript{B} receptors on ON terminals**

Functional GABA\textsubscript{B} receptors are heteromeric complexes of the GABA\textsubscript{B1R} subunit, which exists in two splice variants, R1a and R1b, and the GABA\textsubscript{B2R} subunit (Kaupmann et al. 1998; Kuner et al. 1999). Combination of the GABA\textsubscript{B1R} with the GABA\textsubscript{B2R} appears to be essential for transport to the membrane and full functionality of the receptor (Couve et al. 1998; Kaupmann et al. 1998; White et al. 1998). In the rat OB, the glomeruli have the highest concentration of GABA\textsubscript{B} receptors as determined by radioligand autoradiographic studies (Bower et al. 1987; Chu et al. 1990) and by immunohistochemical localization of the GABA\textsubscript{B1R}a/b subunit (Margeta-Mitrovic et al. 1999). Combined electron microscopy and immunohistochemical staining for GABA\textsubscript{B1R} revealed that the dense labeling of the glomeruli is due to the presence of
GABA<sub>B</sub> receptors on ON terminals and in the somata of periglomerular cells (Bonino et al. 1999). In addition, ORNs in the nasal epithelium label uniformly with antiserum to GABA<sub>A</sub> R1 (Aroniadou-Anderjaska et al. 1999c), suggesting that all ORNs express GABA<sub>B</sub> receptors, which may be transported to their axon terminals. Although it remains to be determined whether ORNs also express the GABA<sub>B</sub> R2 subunit, the functionality of GABA<sub>B</sub> receptors on the axon terminals of the ORNs was suggested by previous studies (Keller et al. 1998; Nickell et al. 1994), and is clearly demonstrated by the present data. Thus it has been shown that baclofen blocks ON-evoked mitral cell spiking (Nickell et al. 1994) and optical signals generated by JG cells (Keller et al. 1998). It was suggested that the dramatic effects of baclofen may imply, at least in part, a presynaptic action (Keller et al. 1998; Nickell et al. 1994). The present study provides solid support of this view, and further shows that baclofen blocks ON input to the OB by a presynaptic action alone. Thus ON-evoked synaptic responses of both glomerular targets of the ON, the M/T cells, and JG cells, were completely suppressed by baclofen, with no concomitant postsynaptic changes in input resistance or holding current. The small hyperpolarization of mitral cells following application of baclofen could be due to blockade of tonic activation of these cells by glutamate release from ON terminals. Additional evidence that baclofen can block ON transmission by acting exclusively on ON terminals comes from the experiment where an NMDA current sink was evoked in the apical dendrites of M/T cells via two independent pathways (ON and LOT stimulation), and only the ON-evoked sink was suppressed by baclofen (Fig. 5).

The mechanism by which baclofen suppressed transmission from the ON appeared to be a reduction in the probability of glutamate release. In a paired-pulse paradigm, the higher the probability of transmitter release during the conditioning pulse, the smaller the response to the test pulse will be (larger PPD) (Debanne et al. 1996; Manabe et al. 1993; Thomson et al. 1993). Baclofen reversed PPD of the ON-evoked responses of M/T cells to PPF (Fig. 4) and reduced depression of the JG cell EPSCs during repetitive ON stimulation (Fig. 6B), effects that are consistent with a decreased probability of glutamate release from ON terminals. As shown in other CNS areas, this could be effected by a reduction of Ca<sup>2+</sup> influx into the terminals (Dolphin 1995; Lambert et al. 1991; Mott and Lewis 1994; Takahashi et al. 1998; Wu and Saggau 1995, 1997), and/or a direct effect on the release process downstream to Ca<sup>2+</sup> influx (Dittman and Regehr 1996; Jarolimek and Misgeld 1997; Scanziani et al. 1992). In turtle olfactory nerve terminals, baclofen was recently shown to reduce Ca<sup>2+</sup> influx (Wachowiak and Cohen 1999).

The present data do not provide evidence for direct effects of baclofen on JG cells or mitral cells, since there were small or no postsynaptic changes when ON-evoked responses of M/T cells and JG cells were completely suppressed by baclofen. In addition, the lack of any effects of baclofen or CGP-35348 on synaptic transmission from M/T cells to granule cells (Fig. 8) suggests that glutamate release from the lateral dendrites of M/T cells is not modulated by GABA<sub>B</sub> receptors. Although both JG cells and mitral cells stain with antiserum to GABA<sub>A</sub>R1a/b (Bonino et al. 1999; Margeta-Mitrovic et al. 1999), there are no cells in the rat OB expressing significant levels of the GABA<sub>B</sub>R2 subunit, as determined by in situ hybridization (Kaupmann et al. 1998). Thus JG cells and mitral cells may not have functional GABA<sub>B</sub> receptors, and the GABA<sub>B</sub> Receptor subunits that these cells express may not be transported to synaptic sites. Consistent with the latter, there is minimal staining for GABA<sub>B</sub>R1a/b in the EPL (Margeta-Mitrovic et al. 1999), where the lateral dendrites of mitral cells receive the vast majority of GABAergic synaptic inputs. In addition, Bonino et al. (1999) did not detect GABA<sub>B</sub>R1 immunoreactivity on dendrites of either JG cells or M/T cells in the glomerular layer. Thus it is possible that in the rat OB only the GABA<sub>B</sub> receptors on ON terminals are fully functional. This, however, requires further investigation.

Tonic and synaptically evoked activation of GABA<sub>B</sub> receptors by endogenous GABA

In the present study, GABA<sub>B</sub> antagonists increased responses of M/T cells evoked by single-pulse ON stimulation, suggesting that ambient levels of extracellular GABA tonically activate GABA<sub>B</sub> receptors on ON terminals. In addition, GABA<sub>B</sub> antagonists reduced the depression of test responses of M/T cells to paired-pulse stimulation of the ON, indicating that a single stimulus pulse to the ON (the conditioning pulse)
elevates extracellular GABA above tonic levels, to further activate GABA\textsubscript{B} receptors on ON terminals. The source of endogenous GABA in the glomeruli of the OB is a subpopulation of JG cells, the PG cells (Gall et al. 1987; Kosaka et al. 1985; Mugnaini et al. 1984; Ribak et al. 1977a). PG cells are activated by ON stimulation (present data and Bardoni et al. 1996; Heyward et al. 1997; Keller et al. 1998); they form dendrodendritic synapses with M/T cells and other JG cells, but they do not form synapses with ON terminals (Hinds 1970; Pinching and Powell 1971b; Ribak et al. 1977a). In most CNS areas, GABAergic neurons do not form synapses with excitatory terminals. However, GABA\textsubscript{B} heteroreceptors

**FIG. 7.** GABA\textsubscript{B} receptors on ON terminals are activated both tonically and in response to ON stimulation: GABA\textsubscript{B} antagonists increase both conditioning and test responses of M/T cells to paired-pulse stimulation of the ON, while reducing PPD of test responses. In all experiments, APV is included in the medium. A: responses to paired-pulse stimulation of the ON (interstimulus intervals 50, 100, 200, 300, and 400 ms) before and after application of CGP-35348 (1 mM). The GABA\textsubscript{B} antagonist increased the amplitude of the conditioning response and reduced or blocked PPD. Each trace is an average of 5 sweeps, and 5 traces are superimposed. B: group data (n = 10) of the effects of CGP-35348 (500 \mu M to 1 mM) on PPD of the ON-evoked glomerular field EPSP. The reduction of PPD was statistically significant (*P \textless 0.05) at interstimulus intervals from 50 to 400 ms. C: time course of the effects of CGP-55845A (10 \mu M) on conditioning and test responses (interstimulus interval 100 ms). Group data from 6 slices. Error bars in B and C are SE.

**FIG. 8.** Baclofen or CGP-35348 do not affect transmission from M/T cells to granule cells. A and B are 2 different slices. A: field potential recorded in the GCL in response to ON or LOT stimulation. When baclofen (50 \mu M) blocked the ON-evoked response, the response evoked by LOT stimulation was unaffected. B: CGP-35348 (1 mM) reduced the depression of the GCL field potential during paired-pulse ON stimulation, but did not affect depression of the GCL field potential in response to paired-pulse stimulation of the LOT. Each trace is an average of 5 sweeps. In the paired-pulse traces, 4 traces are superimposed.
can be activated by low concentrations of extrasynaptic GABA, due to their high affinity for GABA (Sodickson and Bean 1996; Yoon and Rothman 1991). In the hippocampus (Isaacson et al. 1993) and cerebellum (Dittman and Regehr 1997), extrasynaptic GABA reaches sufficiently high levels to activate GABAB heteroreceptors only following high-frequency stimulation of GABAergic neurons. However, tonic activation of GABAB heteroreceptors has been reported in other CNS areas (Emri et al. 1996; Kombian et al. 1996).

The more effective activation of GABAB heteroreceptors in the OB, compared with the hippocampus or cerebellum, may be due to differences between these structures in the proximity of GABA release sites to GABAB heteroreceptors, and/or the proximity of GABA uptake systems to GABA release sites and GABAB heteroreceptors. GABA transporters are located on GABAergic neurons and glia cells (Hertz 1979). In the OB, the dendrodendritic synapses between PG and M/T cells are partially enclosed by glia processes (Chao et al. 1997; Kasowski et al. 1999). Thus GABA released from PG cells at dendrodendritic synapses with M/T cells would probably have to escape the glia barrier to reach GABAB receptors on ON terminals. Although this cannot be excluded, another possibility is that GABA is released from nonsynaptic dendritic sites of PG neurons that are close to the ON terminals. Consistent with this, synaptic vesicles are present in some PG cell dendrites apposed to olfactory nerve terminals that are immunopositive for GABAB receptors (Bonino et al. 1999). In addition, there are only few glial processes close to axodendritic synapses formed by the ON (Chao et al. 1997; Kasowski et al. 1999), which may facilitate the build-up of extrasynaptic GABA. The glomerular inhibitory interactions suggested by the present and previous findings are summarized schematically in Fig. 9.

Relation to the function of the OB

Olfactory information is thought to be encoded by specific patterns of glomerular activity (Buck 1996; Friedrich and Korsching 1997; Guthrie et al. 1993; Johnson and Leon 1996; Johnson et al. 1998; Laurent 1996; Shepherd 1994; Stewart et al. 1979). The present findings suggest that GABAB receptor-mediated presynaptic inhibition of ON input to the glomeruli may play an important role in determining both spatial and temporal components of glomerular activation. Tonic activation of GABAB receptors on ON terminals may serve to filter out weak (“noise”) signals. This may sharpen the spatial pattern of active glomeruli and facilitate detection of the predominant odor. Strong signals are likely to activate more PG cells and evoke stronger inhibition of subsequent ON inputs. This is likely to affect the temporal patterns of glomerular activation in response to repetitive sniffs. During repetitive sniffing, presynaptic GABAB inhibition may adjust the level of glomerular excitation, as a function of sniffing frequency. Our PPD data predict that GABAB presynaptic inhibition will be most effective in inhibiting signals arriving at 100- to 200-ms intervals (5–10 Hz), the dominant frequency during exploratory sniffing (Komisaruk 1970; Macrides and Chorover 1972; Welker 1964). However, a novel odor occurring within such a sniffing bout would activate different glomeruli and, initially, would not be inhibited to the same degree. Thus frequency-dependent GABAB receptor-mediated inhibition of input to the glomeruli may enhance detection of novel stimuli.
The extent to which presynaptic GABA\textsubscript{B} inhibition influences glomerular excitation may vary dynamically, as centrifugal inputs to the glomerulai may alter PG cell excitability (Pinching and Powell 1971c; Shipley et al. 1996). In addition, there is evidence for anatomical connections between glomeruli (Cajal 1911; Pinching and Powell 1971a). If these connections affect GABA release, then GABA\textsubscript{B} receptors on ON terminals could mediate interglomerular influences on glomerular activity patterns. Finally, GABA\textsubscript{B} receptors on ON terminals could play a role in induction of long-term potentiation at the first synapse in the olfactory system (Ennis et al. 1998).

We thank Dr. Nevin A. Lambert for valuable discussions. We also thank Drs. Asaf Keller, Scott M. Thompson, and Michael Meredith for critical review of the manuscript. We are grateful to Dr. Wolfgang Froestl and Novartis Pharma Inc. for the generous gift of the GABA\textsubscript{B} antagonists.

This work was supported by National Institutes of Health Grants DC-00347, DC-03195, DC-02173, and NS-36940. F.-M. Zhou was supported in part by a Young Investigator Award from the National Alliance for Research in Schizophrenia and Depression.

REFERENCES


BOWERY NG, HUTSON AL, AND PRICE GW. GABA\textsubscript{A} and GABA\textsubscript{B} receptor site distribution in the rat central nervous system. *Neuroscience* 20: 365–383, 1987.


KOSAKA T, HATAGUCHI Y, NAGATSU I, AND WU J-Y. Coexistence of immunoreactivities for glutamate decarboxylase and tyrosine hydroxylase in some neurons in the periglomerular region of the rat main olfactory bulb: possible...


