Adrenoceptor-Mediated Elevation of Ambient GABA Levels Activates Presynaptic $GABA_B$ Receptors in Rat Sensorimotor Cortex

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Bennett, Ben D., John R. Huguenard, and David A. Prince. Adrenoceptor-mediated elevation of ambient GABA levels activates presynaptic $GABA_B$ receptors in rat sensorimotor cortex. *J. Neurophysiol.* 78: 561–566, 1997. At inhibitory synapses in the mature neocortex and hippocampus in vitro, spontaneous action-potential-dependent and -independent release of γ-aminobutyric acid (GABA) activates postsynaptic $GABA_A$ receptors but not pre- or postsynaptic $GABA_B$ receptors. Elevation of synaptic GABA levels with pharmacological agents or electrical stimulation can cause activation of $GABA_B$ receptors, but the physiological conditions under which such activation occurs need further elucidation. In rodent sensorimotor cortex, epinephrine produced a depression in the amplitude of evoked monosynaptic inhibitory postsynaptic currents (IPSCs) and a concomitant, adrenoceptor-mediated increase in the frequency of spontaneous IPSCs. Blockade of $GABA_B$ receptors prevented the depression of evoked IPSC amplitude by epinephrine but did not affect the increase in spontaneous IPSC frequency. These data show that adrenoceptor-mediated increases in spontaneous IPSCs can cause activation of presynaptic $GABA_B$ receptors and indirectly modulate impulse-related GABA release, presumably through elevation of synaptic GABA levels.

INTRODUCTION

The inhibitory neurotransmitter γ-aminobutyric acid (GABA) produces the majority of its actions in the brain through activation of two classes of membrane-bound macromolecules, $GABA_A$ and $GABA_B$ receptors (Bormann 1988; Macdonald and Olsen 1994; Mody et al. 1994; Ogata 1990; Sivilotti and Nistri 1991; Thompson 1994). The $GABA_A$ receptor is a ligand-gated ion channel that is selectively permeable to chloride ions (Bormann et al. 1987; Schofield 1989; Schofield et al. 1987), whereas the $GABA_B$ receptor is G protein coupled, and its activation alters potassium (K^+) and/or calcium (Ca^{2+}) conductance (Andrade et al. 1986; Blaxter and Carlen 1985; Bowery 1993; Doze et al. 1995; Gähwiler and Brown 1985; Inoue et al. 1985; Newberry and Nicoll 1984a,b; Ogata et al. 1987). Activation of postsynaptic $GABA_B$ receptors produces K^+-mediated ''slow'' inhibitory events (Alger and Nicoll 1982; Dutar and Nicoll 1988; Otis et al. 1993), whereas presynaptic $GABA_B$ receptors attenuate GABA release by activating K^+ channels and/or decreasing Ca^{2+} conductance (Thompson 1994). GABA is released spontaneously through both action-potential-dependent and -independent mechanisms at inhibitory cortical synapses, causing activation of postsynaptic $GABA_A$ receptors, but not presynaptic or postsynaptic $GABA_B$ receptors, in vitro (Otis and Mody 1992; Thompson and Gähwiler 1992; but see McLean et al. 1996). However, experimental maneuvers that cause elevations in ambient GABA levels, such as blockade of GABA uptake (Isaacson et al. 1993; Thompson and Gähwiler 1992), application of convulsant drugs (Otis and Mody 1992; Scanziani et al. 1991), or electrical stimulation (Otis and Mody 1992), cause activation of $GABA_B$ receptors.

The circumstances that normally lead to such elevations in extracellular GABA and recruitment of presynaptic $GABA_B$ receptors in cortical circuits are unclear. One candidate mechanism might be an increase in spontaneous GABA release. Epinephrine (EPI) increases the frequency of spontaneous inhibitory postsynaptic currents (IPSCs) in neurons of rat sensorimotor cortical slices by activating α-adrenoceptors (Bennett et al. 1995), and concurrently causes a reduction in the amplitude of monosynaptic evoked IPSCs in the majority of neurons tested (see below). We conducted experiments to investigate the possibility that EPI might be producing a depression in evoked IPSC amplitude as a result of α-adrenoceptor-mediated increases in spontaneous IPSCs that elevate synaptic GABA levels and cause activation of presynaptic $GABA_B$ receptors.

METHODS

Standard procedures were used for preparation and recording from slices. Briefly, 400-μm-thick coronal slices through the sensorimotor cortex were prepared from Sprague-Dawley rats of either sex, ages 9–12 days postnatal, in strict accordance with a procedure approved by the Stanford University Animal Use and Care Committee. The slices were cut in a ''low''-calcium, ''high''-magnesium ice-cold saline solution in which 230 mM sucrose had been substituted for NaCl (modified from Aghajanian and Rasmussen 1989). Slices were then transferred to a holding chamber that contained artificial cerebrospinal fluid (ACSF) composed of (in mM) 124 NaCl, 5 KCl, 2 $CaCl_2$, 2 $MgSO_4$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 10 dextrose, pH 7.4 when gassed with 95% O_2-5% CO_2 at 28°C. After incubation for $\geq$1 h, slices were transferred to a recording chamber where they were minimally submerged and maintained at 35 $\pm$ 0.5°C (mean $\pm$ SE). Synaptic currents from layer V neurons were recorded with the use of the ''blind'' whole cell patch-clamp technique (Blanton et al. 1989). The intracellular solution contained (in mM) 120 cesium gluconate, 11 CsCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, 11 ethylene glycol-bis(β-aminoethyl ether)-*N,N,N*′,*N*′-tetraacetic acid, 2 Na-ATP, 0.4 sodium guanosine 5′-triphosphate, 5 *N*′-(2,6-dimethylphenylcarbamoylmethyl) triethylammonium bromide (QX-314, Astra), and 2.5–5 biocytin (Sigma), pH adjusted to 7.3 with CsOH, osmolarity adjusted to 290 mosM with H_2O. Once a whole cell recording was obtained, the slice was perfused with ACSF containing 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione and 50 μM DL-2-aminophosphonovalerate [both from Research Biochemicals International (RBI)]. Neurons were voltage clamped at 0 mV with a List EPC 7 amplifier (Darmstadt, Germany) and recordings wererejected if the access resistance changed by >25% of the initial value or exceeded 20 MΩ (11 ± 3.7 MΩ, mean ± SD; $n = 30$). Monosynaptic IPSCs were evoked by passing constant current pulses through bipolar tungsten electrodes located in layer V, 200–500 μm lateral to the recording electrode. Stimuli were applied at 0.1 Hz and 1.5 times threshold for evoking an IPSC. Synaptic currents were acquired with the use of pClamp version 5.5, filtered at 1–3 kHz and digitized at 44 kHz (DR-484, Neurodata Instruments). The evoked IPSCs were analyzed with the use of METATAPE (J. Huguenard, Stanford University). Spontaneous IPSCs were stored on video tape after digitization and then analyzed off-line with the use of DETECTOR (J. Huguenard). All values are given as means ± SE unless otherwise stated. Baclofen and EPI were obtained from RBI, and *P*-[3-aminopropyl]-*P*-diethoxymethyl phosphinic acid (CGP 35348) was kindly supplied by Dr. Olpe, Ciba-Geigy, Basel, Switzerland. Slices were fixed at the end of each experiment and biocytin-filled neurons were visualized with the use of standard procedures (Horikawa and Armstrong 1988; Tseng et al. 1991).

Rundown of evoked IPSC amplitude was apparent in most of the experiments and on average amounted to 25% of the initial amplitude over 20 min. Control experiments were performed to allow subtraction of this component from estimations of the effect of EPI on evoked IPSCs. During six control recordings, slices were exposed to the vehicle in which EPI was delivered (120 μM ascorbic acid in ACSF), the amount of rundown was assessed, and normalized data from these six cells were combined. The effect of EPI on evoked IPSC amplitude was then calculated by temporally matching the control experiments with those in which EPI was applied. This allowed a more accurate determination of the action of EPI on evoked IPSCs and recovery after drug washout. All data were corrected for rundown before they were pooled. In Figs. 1*D* and 2*E*, neurons were grouped on the basis of comparison with control data; IPSCs in cells in which evoked amplitude was increased or decreased by more than the 95% confidence interval, calculated for pooled data from control neurons, were deemed to be enhanced or depressed, respectively. All other neurons were considered unaffected.

Spontaneous IPSCs were collected (CDR software; J. Dempster, Univ. Strathclyde, Glasgow, UK) and analyzed (DETECTOR) from 2-min recorded segments taken before, during, and after EPI application. These events were then compared with the use of Kolmogorov-Smirnoff nonparametric statistical analysis (K-S test). Groups of events were judged to be significantly different from one another when $P < 0.005$. Pooled data were generated by determining the normalized increase in spontaneous IPSC frequency for each cell and then combining these values to give the mean percentage increase in frequency during EPI application. Values obtained under control conditions, during EPI perfusion, and after washout were compared with the use of the paired *t*-test and differences were considered significant if $P < 0.05$.

Five neurons were exposed to baclofen, and in four of these CGP 35348 was subsequently applied. In three of these instances, slices were exposed to EPI after application of baclofen and CGP 35348. Recordings from six other neurons were obtained from slices that were not exposed to baclofen, but were preincubated with CGP 35348 for ≥5 min before perfusion of EPI. The normalized amplitude of evoked IPSCs was compared between neurons that were exposed to EPI in the absence or presence of CGP 35348, with the use of the unpaired *t*-test, and significance was assigned when $P < 0.05$. The increase in spontaneous IPSCs was also compared between cells exposed to EPI in the presence and absence of CGP 35348 with the use of the unpaired *t*-test, and differences were considered significant if $P < 0.05$.

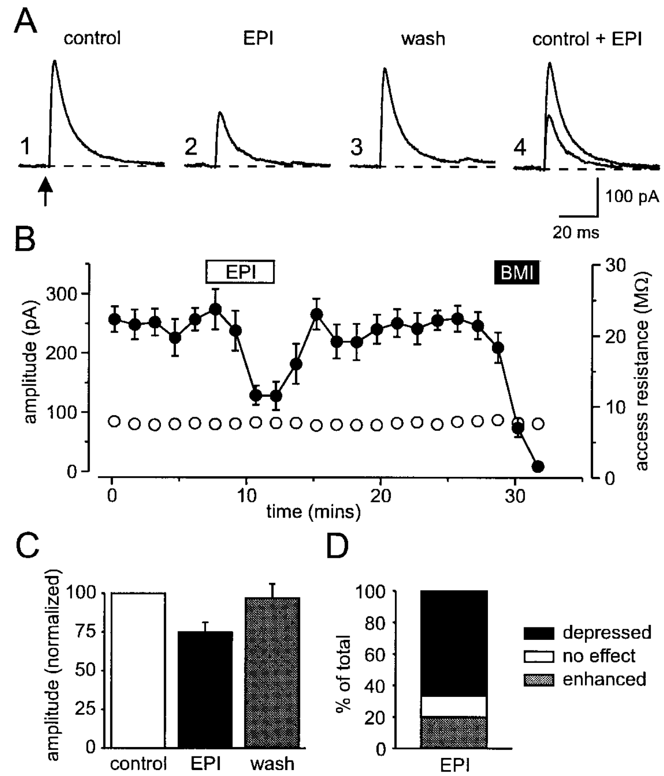

FIG. 1. Epinephrine (EPI) reduces the amplitude of pharmacologically isolated evoked monosynaptic inhibitory postsynaptic currents (IPSCs) in layer V pyramidal neurons. Evoked currents were recorded in the presence of DL-2-aminophosphonovalerate (APV) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). *A*: averages of 12 individual evoked IPSCs, illustrated in control conditions (*1*), after application of 10 μM EPI (*2*), and after a 10-min washout (*3*); and superimposed sweeps from control and EPI conditions (*4*). Stimulus artifacts were blanked in *A* (↑) and in Fig. 2, *A* and *B*. *B*: amplitude of evoked IPSCs vs. time for the same neuron. Averages of 9 consecutive responses (●; mean ± SE) are shown. Bath application of 10 μM EPI reversibly reduced the amplitude of evoked IPSCs. Evoked events were completely blocked by 10 μM bicuculline methiodide (BMI). Horizontal bars above symbols: perfusion of EPI and BMI. Access resistance was stable throughout the experiment (○; *right scale*). *C*: pooled normalized data from 15 neurons illustrate a significant ($P < 0.005$; cf. Fig. 2*D*), reversible depression of evoked IPSC amplitude to 73 ± 8% (mean ± SE) of control. *D*: in the 15 cells of *C*, normalized evoked IPSC amplitude was decreased in 10, unaffected in 2, and enhanced in 3 during EPI application. Dashed lines in *A* and in Fig. 2*A*: baseline current at holding potential of 0 mV; calculated chloride reversal potential = −53 mV for Figs. 1–3.

RESULTS

Recordings were obtained from 30 neurons. All of the biocytin-filled cells that were recovered had the typical morphological features of pyramidal neurons and were located in layer V. Bath application of EPI (10 μM) reversibly reduced the peak amplitude of evoked IPSCs (Fig. 1, *A* and *B*) in the majority (10 of 15) of neurons to a mean value that was 73 ± 8% ($n = 15$) of control (Fig. 1, *C* and *D*). In some neurons, evoked IPSCs were depressed to as much as 40% of control, whereas EPI produced either no effect or an enhancement of IPSC peak amplitude in 5 of 15 neurons (Fig. 1*D*). The input resis-

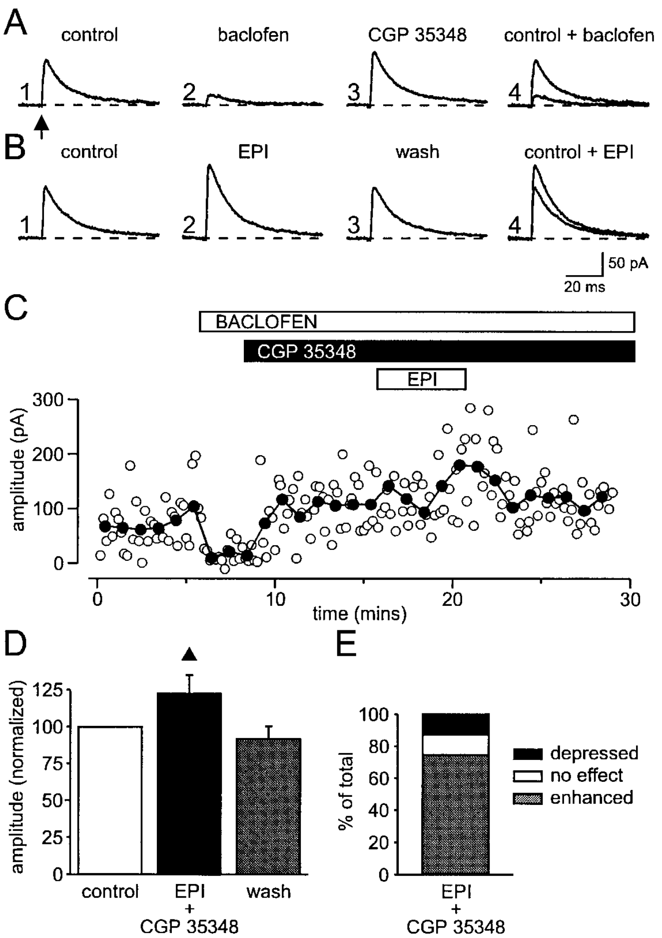

FIG. 2. Blockade of presynaptic γ-aminobutyric acid-B ($GABA_B$) receptors prevents depression of evoked IPSCs by EPI. *A*: averages of evoked monosynaptic IPSCs ($n = 12$) under control conditions in the presence of APV and CNQX (*1*), during application of 10 μM baclofen (*2*), and 5 min after perfusion of 1 mM *P*-[3-aminopropyl]-*P*-diethoxymethyl phosphinic acid (CGP 35348) in the presence of baclofen (*3*); and superimposed sweeps in control and baclofen conditions (*4*). *B*: averages of evoked IPSCs ($n = 12$) under control conditions in the presence of APV, CNQX, baclofen, and CGP 35348 (*1*), in the same solution as in *1* during application of 10 μM EPI (*2*), and after a 5-min washout of EPI (*3*); and superimposed sweeps from control and EPI conditions (*4*). *C*: time series for the experiment from which data in *A* and *B* were generated. Amplitudes of individual evoked IPSCs (○) and averages of 6 adjacent responses (●) are illustrated. Horizontal bars: times of drug perfusion. *D*: pooled normalized data from 9 neurons illustrate that EPI, applied in the presence of CGP 35348, produced a 121 ± 13% increase in the amplitude of evoked IPSCs compared with control cells. There was a significant difference ($P < 0.005$, unpaired *t*-test; ▲) in the effect of EPI application on evoked IPSC amplitude in the presence and absence of CGP 35348 (cf. Figs. 1*C* and 2*D*). *E*: in comparison with control cells, EPI increased the amplitude of evoked IPSCs in 7 of 9 neurons.

tance and postsynaptic response to iontophoretic GABA application were unaltered by application of EPI (Bennett et al. 1995).

To further characterize the actions of EPI, effects on spontaneous IPSCs were examined. Massive increases in the frequency of spontaneous IPSCs accompanied the EPI-induced changes in evoked IPSC amplitude in all 15 cells tested (Fig. 3, *A1–A4*). Statistical analysis of spontaneous events from five neurons revealed that EPI produced significant ($P < 0.005$, K-S test) and reversible increases in spontaneous IPSC frequency (Fig. 3, *A1–A4*) to a mean value of 14.1 ± 3.9 Hz, which was 1,081 ± 303% ($P < 0.05$, *t*-test; $n = 5$) of control (Fig. 3*A4*). There was no consistent effect on the amplitude of spontaneous IPSCs (see DISCUSSION).

To test the hypothesis that adrenoceptor-mediated increases in spontaneous IPSC frequency might produce a decrease in the amplitude of evoked IPSCs through activation of $GABA_B$ receptors, we examined the effect of EPI on evoked IPSC amplitude following blockade of presynaptic $GABA_B$ receptors with the competitive antagonist CGP 35348 (Olpe et al. 1990). The presence of functional presyn-

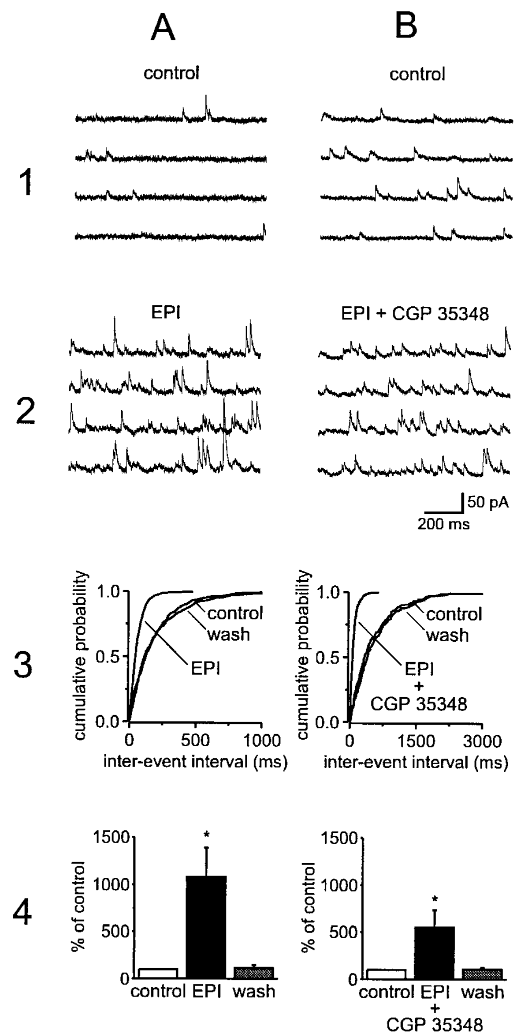

FIG. 3. Blockade of presynaptic $GABA_B$ receptors does not prevent EPI from reversibly increasing the frequency of spontaneous IPSCs. *A1* and *B1*: continuous 4-s recordings of spontaneous IPSCs in control conditions in the presence of APV and CNQX (*A1*) and APV, CNQX, and 0.5 mM CGP 35348 (*B1*). *A2* and *B2*: application of 10 μM EPI produces a very large increase in the frequency of spontaneous IPSCs in the absence (*A2*) and presence (*B2*) of CGP 35348. *A3* and *B3*: cumulative probability plots of interevent intervals for spontaneous IPSCs analyzed from 2-min collection periods before, during, and after application of EPI in the absence (*A3*) and presence (*B3*) of CGP 35348. *A4* and *B4*: pooled normalized data indicate that EPI produced a significant ($P < 0.05$; *t*-test) and reversible increase in the frequency of spontaneous IPSCs to 1,081 ± 303% ($n = 5$) and 552 ± 183% ($n = 5$) of control when applied in the absence (*A4*) and presence (*B4*) of CGP 35348, respectively. The difference between these 2 groups was not significant ($P > 0.05$; *t*-test).

aptic $GABA_B$ receptors was first confirmed by showing that application of baclofen (5 or 10 μM) produced a robust attenuation of evoked IPSC amplitude (16 ± 5% of control, $n = 5$; Fig. 2, *A* and *C*). Concomitant application of CGP 35348 (0.5 or 1 mM) antagonized the effect of baclofen (91± 14% of control, $n = 4$; Fig. 2, *A* and *C*). Slices were then exposed to EPI in the presence of CGP 35348. EPI produced an increase in evoked IPSC amplitude to 121 ± 13% of control in ACSF containing CGP 35348 ($n = 9$), compared with a reduction to 73 ± 8% of control in normal ACSF ($n = 15$). The effect of EPI on evoked IPSC amplitude was significantly ($P < 0.005$, *t*-test) different in the presence and absence of CGP 35348 (cf. Fig. 1, *C* and *D* with Fig. 2, *D* and *E*). Peak evoked IPSC amplitude was reversibly increased by EPI in the presence of CGP 35348 in seven of nine neurons (Fig. 2, *B*, *D*, and *E*). Peak currents were >160% of control in some cells exposed to EPI and CGP 35348, and were unaffected or depressed in two of nine cells (Fig. 2*E*). In the presence of CGP 35348, EPI still produced a significant ($P < 0.005$, K-S test) and reversible decrease in interevent intervals for spontaneous IPSCs in individual neurons (Fig. 3, *B1–B4*). Analysis of pooled data ($n = 5$) showed that adrenoceptor activation in the presence of CGP 35348 caused a 552 ± 183% increase ($P < 0.05$, *t*-test) in the frequency of spontaneous IPSCs (Fig. 3*B4*), which was not different ($P > 0.05$, *t*-test) from the 1,081 ± 303% increase produced by EPI alone.

DISCUSSION

The reversible depression of evoked, monosynaptic IPSC amplitude by EPI in these experiments appears to be due to an indirect effect on presynaptic $GABA_B$ autoreceptors. Activation of adrenoceptors can also alter postsynaptic K^+ conductance (Foehring et al. 1989; Haas and Konnerth 1983; Madison and Nicoll 1982; McCormick and Prince 1988), which could alter the evoked IPSC amplitude. This effect is unlikely to explain the observed effects, however, because postsynaptic K^+ channels were blocked in our experiments by inclusion of cesium (Adelman and French 1978; Hagiwara et al. 1976) and QX-314 (Nathan et al. 1990) in the recording electrode. Also, the input resistance of the recorded neurons was not affected by EPI application (Bennett et al. 1995). Activation of adrenoceptors can also alter Ca^{2+} currents (Gray and Johnston 1987), which might influence the intracellular Ca^{2+} concentration and consequently the phosphorylation state of GABA receptors and their sensitivity to GABA (Chen et al. 1990; Inoue et al. 1986; Stelzer et al. 1988). This also seemed an unlikely explanation for the present data, however, because postsynaptic responses to iontophoretic applications of GABA were unaffected even though EPI produced pronounced effects on the amplitude of evoked IPSCs (Bennett et al. 1995). The blockade of EPI-induced decreases in evoked IPSC amplitude by CGP 35348 also strongly supports the conclusion that presynaptic $GABA_B$ autoreceptors mediate the effect. However, a blockade of adrenoceptors through a nonspecific action of CGP 35348 would also prevent EPI-induced depression of evoked IPSC amplitude. The fact that there was no significant difference between the frequency of spontaneous IPSCs produced by EPI in the presence or absence of CGP 35348 argues against this possibility, and the results of binding studies indicate no interaction between 1 mM CGP 35348 and α_1 adrenoceptors (Olpe et al. 1990). Additionally, in a few cases the increased frequency of spontaneous IPSCs produced by EPI was comparable in either the absence or presence of CGP 35348, even though evoked IPSCs were depressed in the absence and enhanced in the presence of the antagonist. Thus activation of presynaptic adrenoceptors in sensorimotor cortex can modulate evoked monosynaptic GABA currents and thereby alter inhibitory transmission between interneurons and principal cells of layer V. Recently, activation of $GABA_B$ receptors by spontaneous GABA release has been reported in immature hippocampal slices, where synchronous giant GABAergic synaptic events occur under control conditions (McLean et al. 1996).

In previous experiments (Bennett et al. 1995), we found that EPI increased the frequency of spontaneous IPSCs through activation of α-adrenoceptors, an effect that has been described in the hippocampus (Bergles et al. 1996; Doze et al. 1991; Madison and Nicoll 1988). However, in contrast to our findings in neocortex, no decrease inevoked monosynaptic inhibitory postsynaptic potential amplitude was observed in hippocampal CA1 pyramidal neurons exposed to adrenergic agents (Doze et al. 1991; Madison and Nicoll 1988). This discrepancy may be due to several factors such as region-specific or ontogenetic differences in regulation of extracellular GABA levels (Draguhn and Heinemann 1996) or variability in distribution of $GABA_B$ presynaptic receptors (Lambert and Wilson 1993).

Activation of presynaptic $GABA_B$ receptors can produce a profound reduction in the release of GABA (Bowery et al. 1980) and therefore influence the level of inhibition according to the level of activity (Davies et al. 1991; Mott and Lewis 1991; Mott et al. 1993; Thompson et al. 1993). Blockade of $GABA_B$ receptors unmasked an EPI-induced enhancement of evoked IPSC amplitude, but did not prevent the α-adrenoceptor-mediated elevation in the frequency of spontaneous IPSCs. These data indicate that the increased GABA release that occurs as a consequence of the elevation of spontaneous IPSC frequency causes activation of $GABA_B$ receptors, producing a depression of evoked IPSC amplitude. The absence of a similar effect on the amplitude of spontaneous IPSCs would seem to run contrary to this interpretation. However, the amplitude of spontaneous IPSCs was comparable with that of tetrodotoxin-insensitive miniature IPSCs recorded from rats ages 15–18 days postnatal, and the frequency of these miniature IPSCs was largely unaffected by EPI (unpublished observations). Thus spontaneous IPSCs detected in this study are likely to arise from the action-potential-dependent activation of single presynaptic boutons, and an alteration in the amplitude of these events would not be expected to arise following activation of presynaptic $GABA_B$ receptors. The mechanism responsible for the enhancement of the amplitude of evoked IPSCs following EPI application after blockade of $GABA_B$ receptors is unclear. However, a β-adrenoceptor-mediated increase in the amplitude of evoked excitatory postsynaptic currents has been described in the hippocampus (Gereau and Conn 1994).

The present data illustrate that presynaptic $GABA_B$ receptors can be activated during increases in spontaneous IPSC frequency that occur as a consequence of stimulation of endogenous interneuronal receptors, specifically α-adrenoceptors. Functionally, tonic shunting inhibition (Otis and Mody 1992), which has recently been described in cortical pyramidal neurons (Salin and Prince 1996), would be increased by these adrenergic actions and would attenuate the effects of background excitatory inputs by increasing the threshold current required for spike initiation. Simultaneously, attenuation of evoked IPSCs would increase the effectiveness of phasic excitatory events. The consequence of adrenoceptor activation might therefore be an increase in signal-to-noise ratio, as proposed for adrenergic actions at other CNS sites (Madison and Nicoll 1986; Moore and Bloom 1979).

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