

Adrenergic Modulation of GABA_A Receptor-Mediated Inhibition in Rat Sensorimotor Cortex

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Bennett, B. D., J. R. Huguenard, and D. A. Prince. Adrenergic modulation of GABA_A receptor-mediated inhibition in rat sensorimotor cortex. *J. Neurophysiol.* 79: 937–946, 1998. The effect of adrenoceptor activation on pharmacologically isolated monosynaptic inhibitory postsynaptic currents (IPSCs) detected in layer V pyramidal neurons was examined by using whole cell voltage-clamp in a slice preparation of rat sensorimotor cortex. Epinephrine (EPI; 10 μ M) reversibly altered the amplitude of evoked IPSCs (eIPSCs) in slices from postnatal day 9–12 (P9–12) and P15–18 rats. The effects of EPI were heterogeneous in both age groups, and in individual cases an enhancement, a depression or no effect of eIPSCs was observed, although depression was observed more commonly in the younger age group. The effects of EPI on eIPSC amplitude were likely mediated through presynaptic mechanisms because they occurred in the absence of any alteration in the current produced by direct application of γ -aminobutyric acid (GABA), or in input resistance. EPI always elicited an increase in the frequency of spontaneous IPSCs (sIPSCs) irrespective of whether or not it induced any change in the amplitude of eIPSCs in the same neuron. The increase in sIPSC frequency was blocked by phentolamine (10 μ M) but not by propranolol (10 μ M), supporting the conclusion that EPI-mediated effects on sIPSC frequency result from activation of α -adrenoceptors located on presynaptic inhibitory interneurons. In a subpopulation of neurons (3/9) from P15–18 rats, EPI increased both the amplitude and frequency of miniature IPSCs (mIPSCs) recorded in the presence of tetrodotoxin (TTX) and under conditions where postsynaptic EPI effects were blocked, suggesting activation of adrenoceptors on presynaptic terminals in these cells. Results of these experiments are consistent with an action of EPI at adrenoceptors located on presynaptic GABAergic interneurons. Adrenergic activation thus has multiple and complex influences on excitability in cortical circuits, some of which are a consequence of interactions that regulate the strength of GABAergic inhibition.

INTRODUCTION

Inhibitory neurotransmission mediated by γ -aminobutyric acid (GABA) plays a vital role in the integration of synaptic signals by individual neurons and regulation of excitability within neuronal circuits of cortical structures in the CNS. For example, GABAergic mechanisms are involved in the control of receptive field properties within the visual system (Bolz and Gilbert 1986; Hata et al. 1988; Sillito 1977, 1979), regulation of inter- and intracolumnar excitability (Chagnac-Amitai and Connors 1989; Salin and Prince 1996), control of action-potential generation (Kim et al. 1995; Wong and Prince 1979), modulation of transmitter release at presynaptic terminals (Isaacson et al. 1993; Morishita and Sastry 1994; Morrisett et al. 1991; Thompson et al.

1993), and generation of oscillatory rhythms (Buzsaki and Chrobak 1995; Michelson and Wong 1994).

The noradrenergic innervation of the neocortex and hippocampus is also important in regulating excitability of individual cells, synaptic transmission and network function. Activation of β -adrenoceptors depolarizes pyramidal neurons (Foehring et al. 1989; Madison and Nicoll 1986) and reduces slow afterhyperpolarizations, thereby altering spike frequency accommodation (Dodt et al. 1991; Foehring et al. 1989; Lorenzon and Foehring 1993; Madison and Nicoll 1982, 1986). Norepinephrine can also affect both excitatory and inhibitory synaptic transmission through activation of presynaptic adrenoceptors (Dodt et al. 1991; Doze et al. 1991; Gereau and Conn 1994; Madison and Nicoll 1988; Scanziani et al. 1993). A modulation of GABAergic inhibition mediated by α -adrenoceptors was shown in hippocampus, where norepinephrine indirectly reduces the amplitude of inhibitory postsynaptic potentials (IPSPs) (Doze et al. 1991; Madison and Nicoll 1988) by decreasing the excitatory input onto interneurons (Doze et al. 1991). The effect of adrenoceptor activation on inhibitory transmission in the neocortex has not been investigated, but is an important issue, in light of the extensive cortical innervation by noradrenergic axons (Morrison et al. 1978, 1981; Parnavelas and Papadopoulos 1989), possible involvement in pathophysiological processes such as epileptogenesis (Levit and Noebels 1981; Trottier et al. 1994) and potential role in cortical plasticity (Gordon et al. 1988; Stanton and Sarvey 1985).

This study was undertaken to determine how activation of adrenoceptors might influence neocortical inhibitory synaptic function during the early postnatal period. Some of these data were presented in abstract form (Bennett et al. 1995, 1996).

METHODS

Slice preparation

Experiments were performed in vitro on slices of parietal cortex, area 1 (Paxinos and Watson 1986), prepared from postnatal day 9–12 (P9–12) and P15–18 Sprague-Dawley rats of either sex. The tissue was prepared in accordance with a protocol approved by the Stanford University Animal Use and Care Committee. Animals were deeply anesthetized with pentobarbital sodium (50 mg/Kg) and decapitated and their brains were rapidly removed. Four hundred μ m-thick coronal or horizontal cortical slices were cut with a vibratome (TPI) at 4°C in a modified Ringer solution in which 252 mM sucrose was substituted for 126 mM NaCl (Aghajanian and Rasmussen 1989; Fukuda and Prince 1992). Horizontal

slices were taken at a sufficient depth from the dorsal surface to include all layers of the parietal cortex. The slices were incubated at 30°C in artificial cerebrospinal fluid (ACSF) containing (in mM) 124 NaCl, 5 KCl, 1.25 NaH₂PO₄, 2 CaCl₂, 2 MgSO₄, 26 NaHCO₃, and 10 dextrose, pH 7.4 when gassed with 95% O₂-5% CO₂. Recordings were not made until at least 1 h after the slicing procedure.

Electrophysiological recording

Individual slices were transferred to the recording chamber where they were perfused (1.5 ml/min) with pregassed ACSF and maintained at 35°C. In all experiments, (\pm)-2-amino-5-phosphonopentanoic acid (APV; 50 μ M) and either 6,7-dinitroquinoxaline-2,3-dione (DNQX; 20 μ M) or 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10 μ M) were added to the ACSF to block ionotropic glutamate receptors. Patch electrodes were pulled from thin-wall borosilicate glass tubing (WPI, Sarasota, FL) on a Narishige PP-83 pipette puller (Narishige, Japan) and had resistances of 3–5 M Ω when filled with a solution containing (in mM) 120 Cs gluconate, 11 CsCl, 1 MgCl₂, 1 CaCl₂, 10 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 11 ethylene glycol-bis(β -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), 2 Na-ATP, 0.4 Na-guanosine 5'-triphosphate (Na-GTP), and 5 QX-314. The pH was adjusted to 7.3 with CsOH and osmolarity to 290 mosM with H₂O. Recordings were made with the "blind" whole cell technique (Blanton et al. 1989). Layer V neurons were voltage-clamped at 0 mV with a List EPC7 amplifier (Darmstadt, Germany). Series resistance (5–18 M Ω) was monitored throughout the recording and cells were rejected if values changed by more than 25% or exceeded 20 M Ω . Synaptic currents were evoked by passing constant current pulses through bipolar tungsten stimulating electrodes placed in layer V, 200–500 μ m to either side of the recording electrode. Low frequency (0.1 Hz), 100- μ s pulses were applied and their intensity increased until threshold was reached for eliciting just-detectable monosynaptic IPSCs (typically 50–100 μ A). Stimulus pulses were then increased to 150 μ s duration (1.5 times threshold) and maintained at this intensity for the entire experiment. Correction for the liquid junction potential (12 mV) was made throughout. The Cl⁻ equilibrium potential under these conditions (-53 mV) was calculated by using the Nernst equation, with an activity coefficient correction for extracellular [Cl⁻].

In a few experiments iontophoretic application of GABA was used. Theta glass pipettes were pulled on a vertical Narishige puller (Narishige, Japan) and the tips broken back under microscopic guidance to a diameter of 0.5–1 μ m. One barrel was filled with NaCl (1 M) for current balancing and the second barrel was filled with GABA (1 M, pH 7.3), which was ejected periodically (10 s, 20–100 nA, 0.0167 Hz). Holding currents ranged from 10 to 30 nA. GABA-evoked currents of a constant amplitude could be elicited when the iontophoretic electrode was positioned within 100 μ m of the recording electrode.

Data analysis

Currents were low-pass filtered at 1–3 kHz, digitized with an A/D convertor and analyzed with pClamp v 5.5 software. Spontaneous currents were stored on magnetic videotape after digitization with a PCM convertor (Neurodata Instrument, NY) and analyzed with Scan v 4.0 (J. Dempster, Strathclyde University) or Detector v 3.3 software (Ulrich and Huguenard 1996). The frequency of spontaneous IPSCs (sIPSCs) was quite variable in control periods and was 8.7, 16.6, and 21.9 Hz in different experiments. No direct comparison of the frequency of sIPSCs and miniature IPSCs (mIPSCs) in individual neurons was examined in this study.

The peak amplitude of evoked IPSCs (eIPSCs) was measured after each stimulus and an average obtained for a 2 min control period immediately before drug application, during the last 2 min

of epinephrine (EPI) perfusion and 10 min after application (see Fig. 2). A slight run down of eIPSCs was apparent in all recordings and control experiments were performed to allow separation of EPI-induced effects from run down. During control experiments, the above protocol was used, except that the slices were exposed to the vehicle (120 μ M ascorbic acid in ACSF) in the absence of EPI. The time-dependent run down of eIPSC amplitude during these experiments was monitored and used for constructing control plots to allow separation of adrenoceptor-dependent and -independent effects (see Fig. 3). The amplitude of eIPSCs was normalized for each experiment by dividing the peak current of individual eIPSCs by the average peak IPSC during the 2 min control period. Alterations in the mean eIPSC amplitude during the final two minutes of 5 min EPI applications, which exceeded the 95% confidence interval, calculated for control cells during the equivalent period, were assumed to be a result of adrenoceptor activation. Neurons were then grouped according to whether or not the eIPSC was enhanced, depressed, or unaltered after exposure to EPI. Differences between groups of neurons were considered statistically significant if $P < 0.05$ (unpaired *t*-test). Results are given as means \pm SE. The reversibility of these effects was determined by comparing 2 min samples from drug and vehicle control groups after 10 min of wash out.

mIPSCs were recorded after 5 min of perfusion with ACSF containing 1 μ M tetrodotoxin (TTX) in addition to CNQX/APV as above. sIPSCs and mIPSCs were analyzed from sample periods that were equivalent to those used for eIPSCs. By using Detector, events were discriminated from baseline noise by setting a minimum threshold on the differentiated current trace. Parameters were set to detect sIPSCs or mIPSCs that were above the noise (>7 pA in amplitude). As the frequency and amplitude of sIPSCs and mIPSCs are not normally distributed, a nonparametric analysis (Kolmogorov-Smirnov test; KS-test) was used to determine significance between control, drug, and wash groups, with $P < 0.005$ as the criterion for significant differences between conditions.

Reagents

All drugs were bath applied by dissolving them in ACSF to the final desired concentration. Epinephrine bitartrate (EPI), (\pm)-propranolol hydrochloride, phentolamine mesylate, (-)-bicuculline methiodide (BMI), DNQX, CNQX, APV and TTX citrate were all obtained from Research Biochemicals International. A stock solution of EPI was made fresh in purified water containing ascorbic acid (120 μ M final concentration) before each application and was used at a final concentration of 10 μ M throughout. Propranolol and phentolamine were also made fresh daily in purified water. All other drugs were made up as stock solutions and frozen for storage, then thawed and diluted in ACSF on each experimental day.

RESULTS

Monosynaptic eIPSCs are GABA_A receptor-mediated events

To study the effects of adrenoceptor activation on isolated IPSCs, excitatory transmission was pharmacologically blocked by bathing the slices in ACSF containing CNQX (10 μ M) or DNQX (20 μ M) and APV (50 μ M). Postsynaptic GABA_B receptor-activated K⁺ channels were blocked by Cs⁺ and QX-314 that were present in the patch electrode solution (Adelman and French 1978; Hagiwara et al. 1976; Nathan et al. 1990). Stimulation of layer V (0.1 Hz in all experiments) adjacent to the recording electrode elicited IPSCs in layer V neurons. At a holding potential of \sim 0 mV,

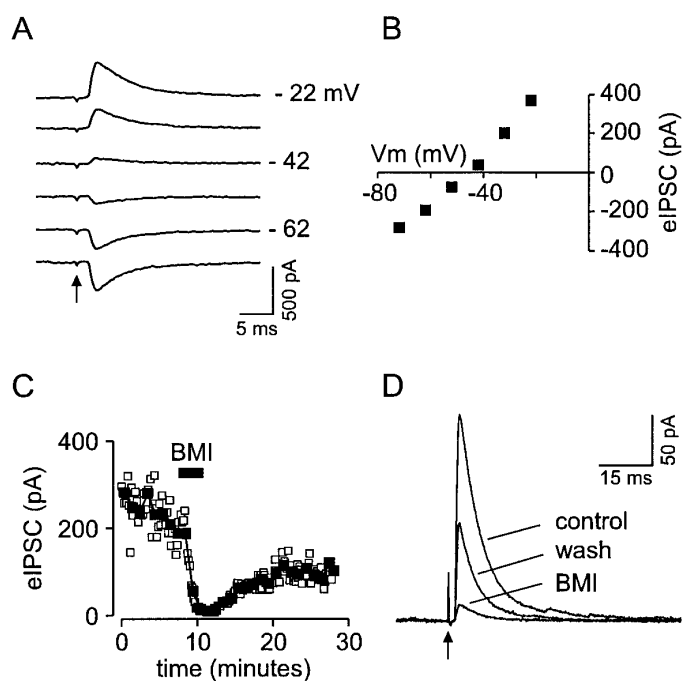


FIG. 1. Evoked IPSCs (eIPSCs) are γ -aminobutyric acid ($GABA_A$) receptor-mediated events. All data are from same neuron. *A* and *B*: current-voltage relationship of eIPSCs. *A*: averaged IPSCs elicited at different holding potentials between -72 and -22 mV. *B*: each point (\blacksquare) represents mean peak current evoked at each potential. Reversal potential for eIPSCs in this neuron was -48 mV, which was close to calculated chloride equilibrium potential of -53 mV (see METHODS). *C* and *D*: bath application of $10 \mu M$ bicuculline methiodide (BMI) reversibly blocked eIPSCs. *C*: amplitudes of individual eIPSCs (\square) are plotted as well as local averages of 6 responses (\blacksquare). *D*: 3 superimposed traces are averages of 6 sweeps taken before (control), during (BMI), and after (wash) bath application of bicuculline. Residual current evoked in presence of $10 \mu M$ BMI was eliminated by application of $30 \mu M$ BMI (not shown). *A* and *D*, \uparrow : stimulus artifact.

the monosynaptic eIPSCs had an average peak amplitude of 277 ± 37 pA ($n = 27$) in response to 1.5 times threshold stimuli. The eIPSCs were dependent on the membrane potential and reversed at -48 mV, close to the Cl^- equilibrium potential of -53 mV (Fig. 1, *A* and *B*). The reversal potential for eIPSCs was the same in slices from P9–12 and P15–18 animals. The eIPSCs were reversibly attenuated by bath application of BMI ($10 \mu M$; Fig. 1, *C* and *D*) and were completely blocked by a higher concentration of bicuculline ($30 \mu M$; not shown). The eIPSCs had a constant latency and a single peak over a range of stimulation intensities (not shown).

Adrenoceptor activation can modulate the amplitude of monosynaptic eIPSCs

The effect of EPI on eIPSCs was investigated in slices from P9–12 and P15–18 rats. The most common response to bath application of EPI was a reversible depression of the amplitude of monosynaptic eIPSCs in neurons recorded in P9–12 slices (Figs. 2, *A* and *B*, and 4*A*) and an enhancement of eIPSCs was more commonly observed in P15–18 neurons (Figs. 2, *C* and *D*, and 4*B*). The decrease or increase of the amplitude of eIPSCs occurred in the absence of any effect on the kinetics of the evoked events (Fig. 2, *B* and *D*, insets) and were partially reversible after a 10–15 min

wash out of the drug (Fig. 2, *A* and *B*). However, slow run down of eIPSC amplitude was observed in all experiments (e.g., Figs. 2*B* and 3). To isolate the effect of EPI from run down of the eIPSC, control data were collected and compared with those obtained during EPI applications (see METHODS). Figure 3 illustrates that there was run down of eIPSCs

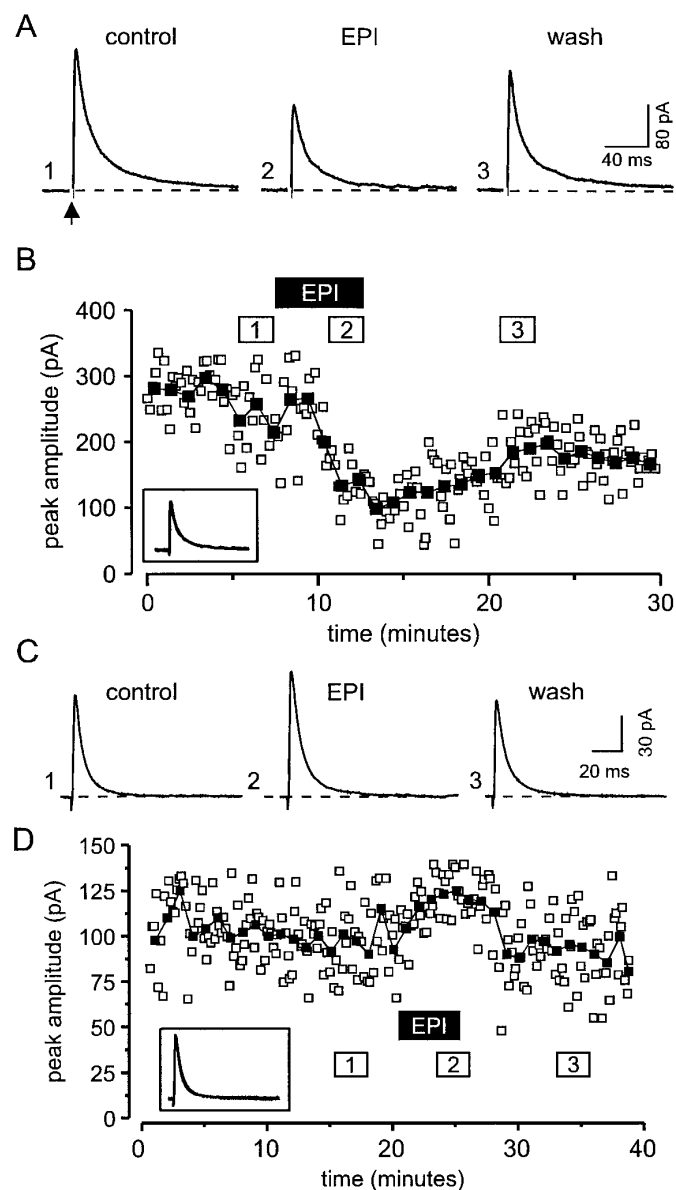


FIG. 2. Epinephrine (EPI) perfusion results in a reversible depression or enhancement of eIPSCs recorded from a layer V neurons. *A* and *B*: depression of eIPSCs in a slice taken from a postnatal day 12 (P12) rat. *A*: each trace represents average of 12 eIPSCs before (1), during (2), and after (3) application of EPI ($10 \mu M$). Number to left of each trace indicates corresponding sample period marked by numbered open rectangles in *B*. Stimulus artifact was blanked from each trace (\uparrow). *B*: individual eIPSCs (\square) and local averages of 6 adjacent points (\blacksquare) as in Fig. 1. In both *A* and *B* amplitude of eIPSCs does not fully recover after EPI application because of run down during course of experiment (see Fig. 3). *C* and *D*: enhancement of eIPSCs in a slice taken from a postnatal day 15 (P15) rat. *C*: averaged eIPSCs before, during, and after EPI application. *D*: raw data depicting individual eIPSC amplitudes (\square), and local averages (\blacksquare) during EPI application. *B* and *D*: insets are superimposed scaled traces of control and EPI responses from *A*1 and *A*2 and *C*1 and *C*2, respectively. Note difference in calibration in *A* and *B* vs. *C* and *D*.

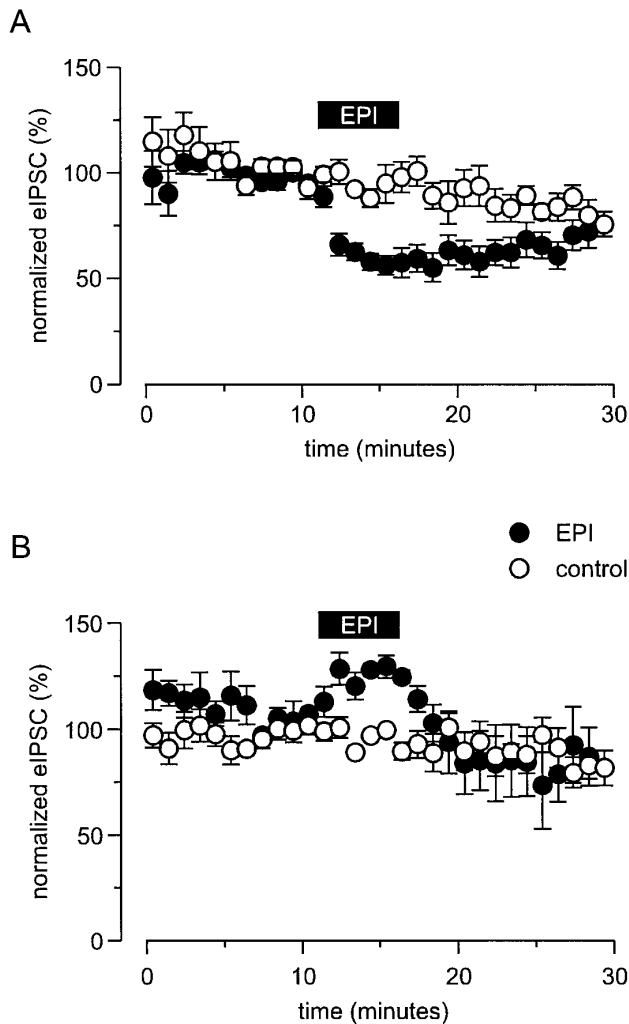


FIG. 3. Examples of EPI-induced decrease (A) or increase (B) in eIPSC amplitude in neurons from P9–12 and P15–18 rats, respectively. *A*: average normalized eIPSC of 10 P9–12 neurons (●) before, during, and after a 5 min exposure to 10 μ M EPI. ○: average normalized eIPSC of 6 P9–12 control neurons that were exposed to vehicle (120 μ M ascorbic acid) in which EPI was delivered. *B*: average normalized eIPSC of 4 P15–18 neurons (●) before, during, and after a 5 min bath application of EPI (10 μ M) that elicited an enhancement of eIPSC amplitude. ○: average normalized eIPSC of 4 P15–18 control neurons exposed to vehicle in which EPI was delivered. During washout of EPI, amplitude of eIPSCs after depression (A) or enhancement (B) shows full recovery when compared with control neurons.

during the course of an experiment, irrespective of whether or not the slices were exposed to EPI or to the vehicle (120 μ M ascorbic acid in ACSF) in which EPI was delivered. However, the EPI effect was clearly distinguishable from the run down control. Data from control experiments were used to demonstrate that adrenoceptor activation produced reversible alterations in the amplitude of eIPSCs, independent of run down (see METHODS).

Adrenoceptor activation produces heterogeneous effects on the amplitude of eIPSCs

Application of EPI produced an enhancement, depression or no effect on eIPSC amplitude in slices from both age

groups (Fig. 4). The relative proportion of neurons exhibiting enhancement or depression of eIPSCs appeared to be altered during the period of postnatal development under study (Fig. 4). For example, a reversible increase in raw eIPSC amplitudes was apparent in only a small fraction (2 of 15) of P9–12 neurons (Fig. 4A, shaded area) and depression was observed in most cases, whereas an increase was seen in greater than half (7 of 12) neurons in P15–18 slices (Fig. 4B, shaded area). Although visual inspection of Fig. 4A indicates that two neurons exhibited an obvious enhancement in eIPSC amplitude, after correction for run down and subsequent statistical analysis (see METHODS) a third neuron also showed a significant enhancement after EPI application. In 66% (10/15) of neurons from P9–12 slices, EPI elicited a significant (unpaired *t*-test; $P < 0.05$) and reversible reduction ($41 \pm 7\%$) in the amplitude of eIPSCs (Fig. 3A). In the same age group, 20% (3/15) of cells were found in which a significant (unpaired *t*-test; $P < 0.05$) reversible enhancement ($32 \pm 4\%$) of eIPSC amplitude could be de-

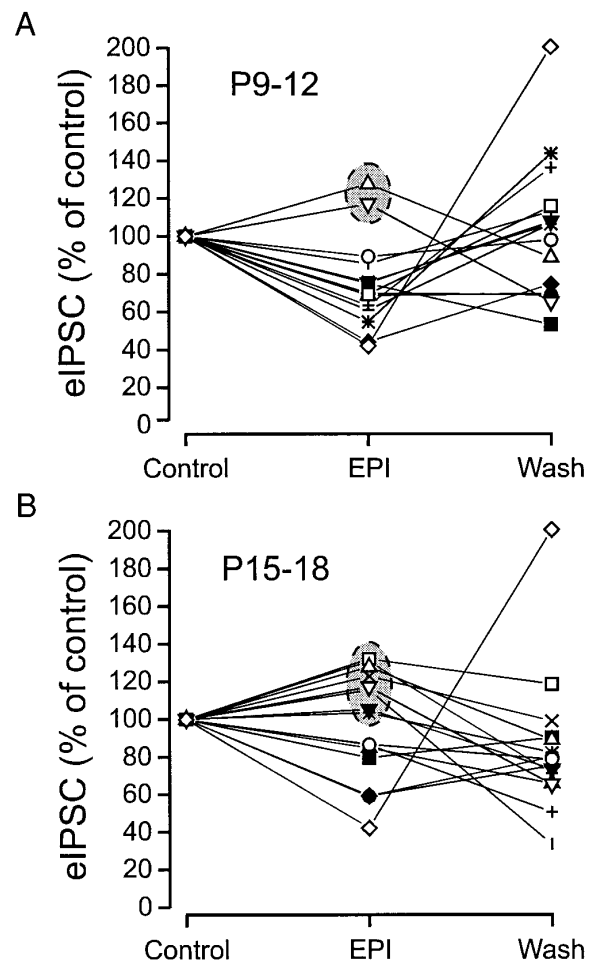


FIG. 4. Adrenoceptor activation produces heterogeneous effects on amplitude of eIPSCs. *A*: in most cases (12/15) with neurons from P9–12 animals, EPI (10 μ M) elicited a reversible depression of eIPSC peak amplitude. In only 2 cases (shaded oval) was an enhancement observed. Each symbol represents data from a single neuron. *B*: by contrast, in neurons from P15–18 animals, EPI (10 μ M) commonly (shaded oval, 7 of 12 cells) caused an increase in eIPSC amplitude and in only four cases was a reversible depression obtained. In this figure eIPSC values were normalized to control levels, but not corrected for rundown.

tected (Fig. 4A). In the remaining 13% (2/15) of neurons, eIPSCs were unaffected by adrenoceptor activation (Fig. 4A). In slices from P15–18 animals, there was an equal proportion (33%, 4/12 neurons in each case; Fig. 4B) of cells in which eIPSCs were significantly enhanced (unpaired *t*-test; $P < 0.05$) (Figs. 2, C and D, and 3B), reduced, or unaffected by EPI. The magnitude of the reduction ($40 \pm 11\%$) and enhancement ($34 \pm 3\%$) of eIPSC amplitude in slices from P15–18 animals was similar to that found in younger tissue.

EPI application did not significantly alter the neuronal input resistance (R_n) of recorded neurons. In slices from P9–12 rats, R_n in control conditions ($156 \pm 38 \text{ M}\Omega$, $n = 15$) was not significantly different from R_n during EPI application ($132 \pm 24 \text{ M}\Omega$, $n = 15$; $P > 0.5$, paired *t*-test). Analysis of the group of cells that displayed a depression of eIPSCs also failed to reveal any alterations in R_n . The R_n in control ($137 \pm 24 \text{ M}\Omega$, $n = 10$) and during EPI perfusion ($135 \pm 26 \text{ M}\Omega$, $n = 10$) were not different from each other ($P > 0.5$; paired *t*-test). In P15–18 slices, R_n values were analyzed both as one group and after separation into groups according to the change in eIPSC amplitude produced by EPI application. There were no differences ($P > 0.5$; paired *t*-test) between control periods and EPI perfusion. Additionally, comparison between neurons from P9–12 and P15–18 slices revealed that there was not a difference ($P > 0.5$; unpaired *t*-test) between R_n values for the two groups (P9–12: $156 \pm 38 \text{ M}\Omega$, $n = 15$; P15–18: $170 \pm 67 \text{ M}\Omega$, $n = 12$).

Adrenoceptor-mediated modulation of eIPSC amplitude is presynaptic

To determine whether or not the above effects of EPI on eIPSCs might be the result of changes in postsynaptic response to GABA, we examined the effect of EPI on eIPSCs and currents evoked by GABA iontophoresis simultaneously in the same neurons. An iontophoretic electrode loaded with GABA was placed close to the soma of the patched neuron and GABA was applied (10 s; 0.0167 Hz; 20–100 nA) to the cell before, during, and after application of EPI. The GABA ejection was adjusted to evoke outward currents comparable in amplitude with eIPSCs ($126 \pm 15 \text{ pA}$, $n = 7$). GABA-evoked currents were unaffected by EPI. Representative results in a neuron from a P17 animal are illustrated in Fig. 5. The amplitude of eIPSCs was increased after EPI application (Fig. 5A) but the iontophoretically evoked current was unaffected (Fig. 5B). Both the eIPSCs and the current elicited by GABA iontophoresis were sensitive to bath application of BMI, indicating that both currents were a result of activation of GABA_A receptors (Fig. 5, A and B). In each of the seven neurons tested, there was no observable effect on the current elicited by iontophoretic GABA application, irrespective of age or whether or not eIPSCs were enhanced, depressed, or unaffected. Overall, the amplitude of the outward current evoked by GABA pulses was reduced by $3 \pm 2\%$ ($n = 4$) in P9–12 slices and by $3 \pm 3\%$ ($n = 3$) in P15–18 slices.

EPI increases the frequency of sIPSCs

Increases in sIPSC frequency were observed after every application of EPI, irrespective of the action of EPI on eIPSC

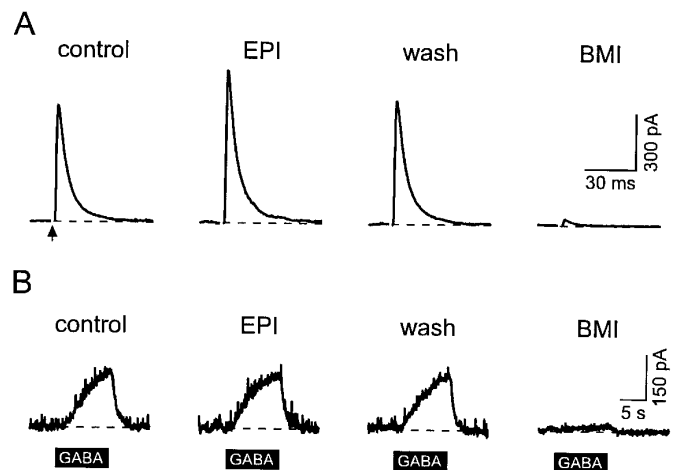


FIG. 5. Adrenoceptor activation does not alter postsynaptic GABA_A receptor function. *A*: reversible increase in eIPSC amplitude after exposure to EPI ($10 \mu\text{M}$) in a neuron from a P17 animal. Data were sampled as described for Fig. 2. Application of BMI ($10 \mu\text{M}$) eliminated eIPSCs. *B*: currents elicited by direct iontophoretic application (10 s, 0.016 Hz, 20 nA) of GABA to recorded neuron (same cell as *A*) were unaffected by EPI ($10 \mu\text{M}$) and blocked by BMI ($10 \mu\text{M}$).

amplitude; these effects are illustrated for a representative P17 neuron in Fig. 6A (cf. control, EPI, and wash). The addition of BMI ($10 \mu\text{M}$) blocked the sIPSCs indicating that they were GABA_A receptor-mediated events (Fig. 6A, BMI). Events were analyzed during the same 2 min periods used for analysis of eIPSCs (see above). Cumulative probability plots indicated that adrenoceptor activation caused a decrease in interevent interval (Fig. 6B) and increase in peak current (Fig. 6C). Statistical analyses using the K-S test revealed that these changes were significant ($P < 0.005$) and reversible (not shown). A total of 10 neurons, 5 from each age group, were analyzed by using K-S statistics to determine the effects of adrenoceptor activation on sIPSC frequency and amplitude. Application of EPI produced a significant ($P < 0.005$, K-S test) increase in frequency of sIPSCs in all cells (10/10; Fig. 6, D and E). The mean frequency of sIPSCs in P9–12 and P15–18 slices before EPI application was $1.3 \pm 0.9 \text{ Hz}$ and $8.7 \pm 2.6 \text{ Hz}$, respectively. These values increased to $7.7 \pm 2.9 \text{ Hz}$ and $21.5 \pm 4.9 \text{ Hz}$, respectively, after adrenoceptor activation.

The action of EPI on sIPSC amplitude was uniform in slices from P15–18 animals where a significant ($P < 0.005$, K-S test) and reversible increase was observed in all neurons (5/5). EPI produced heterogeneous effects on sIPSC amplitude in slices from P9–12 rats. In 2/5 neurons there was a significant ($P < 0.005$) and reversible alteration in sIPSC amplitude with 1 cell displaying an increase and 1 cell displaying a decrease. The remaining 3/5 neurons were unaffected. The mean amplitude of sIPSCs before EPI application was $27.8 \pm 2.6 \text{ pA}$ and $22.0 \pm 2.1 \text{ pA}$ for P9–12 and P15–18 slices, respectively.

Increase in sIPSC frequency by EPI is the result of activation of α -adrenoceptors

Because all neurons exhibited an increase in sIPSC frequency after EPI application, the receptor type underlying this increase was determined by bathing slices from P9–

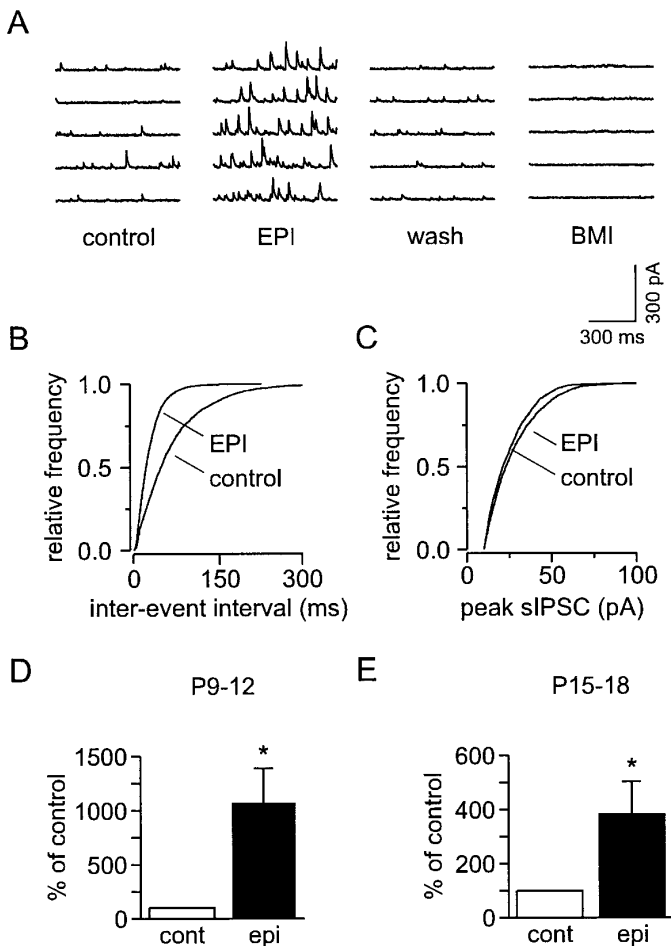


FIG. 6. Effects of EPI on frequency and amplitude of spontaneous IPSCs (sIPSCs). Data in *A* and *B* are from neuron analyzed in Fig. 5, *A* and *B*. *A*: each trace represents a continuous 4-s recording. Application of EPI (10 μ M) produced a reversible increase in frequency of sIPSCs and BMI (10 μ M) blocked sIPSCs. *B* and *C*: cumulative probability plots indicate that EPI produced a significant ($P < 0.005$, K-S test) decrease in interevent interval (*B*) and a significant increase in amplitude of sIPSCs compared with control (*C*). *D* and *E*: EPI produced a significant ($P < 0.005$, K-S test) and reversible (not shown) increase in sIPSC frequency in all neurons examined from both P9–12 slices (*D*; $n = 5$) and P15–18 slices (*E*; $n = 5$) to $1,081 \pm 303$ and $383 \pm 119\%$ of control, respectively.

12 and P15–18 animals in either an α - or β -adrenoceptor antagonist before exposure of the slice to EPI. Preincubation of slices with the β -adrenoceptor antagonist, propranolol (10 μ M), for 10 min before application of EPI did not prevent a reversible increase in sIPSC frequency similar to that seen in the absence of the antagonist (cf. Fig. 6*A* and Fig. 7*A*). Of five neurons that were exposed to EPI after preincubation with propranolol, 4/5 exhibited a significant ($P < 0.005$, K-S test) increase in sIPSC frequency (e.g., the neuron of Fig. 7, *A* and *C*). The mean frequency of sIPSCs was 16.6 ± 6.4 Hz before EPI application and increased to 25.3 ± 9.2 Hz after presumed α -adrenoceptor activation ($n = 5$). Effects on the amplitude of sIPSCs were more heterogeneous. Only 2/5 neurons displayed a significant ($P < 0.005$; K-S test) increase in mean amplitude of sIPSCs when EPI was applied after preincubation with propranolol. Application of EPI, after a 10 min preincubation of slices with the α -adrenoceptor antagonist, phentolamine (10 μ M), was ineffective

in producing increases in the frequency or amplitude of sIPSCs in 4/5 neurons (Fig. 7, *B* and *D*). The mean frequency of sIPSCs under these conditions was 21.9 ± 10.4 and 20.9 ± 10.4 Hz before and after EPI exposure, respectively. The R_n values for neurons preincubated with either propranolol or phentolamine were similar to those given above and were not significantly altered during exposure to EPI ($P > 0.5$; paired *t*-test).

Adrenoceptor activation increases the frequency and amplitude of mIPSCs in a subpopulation of neurons

To further characterize the location of adrenoceptors influencing inhibitory transmission in layer V of sensorimotor

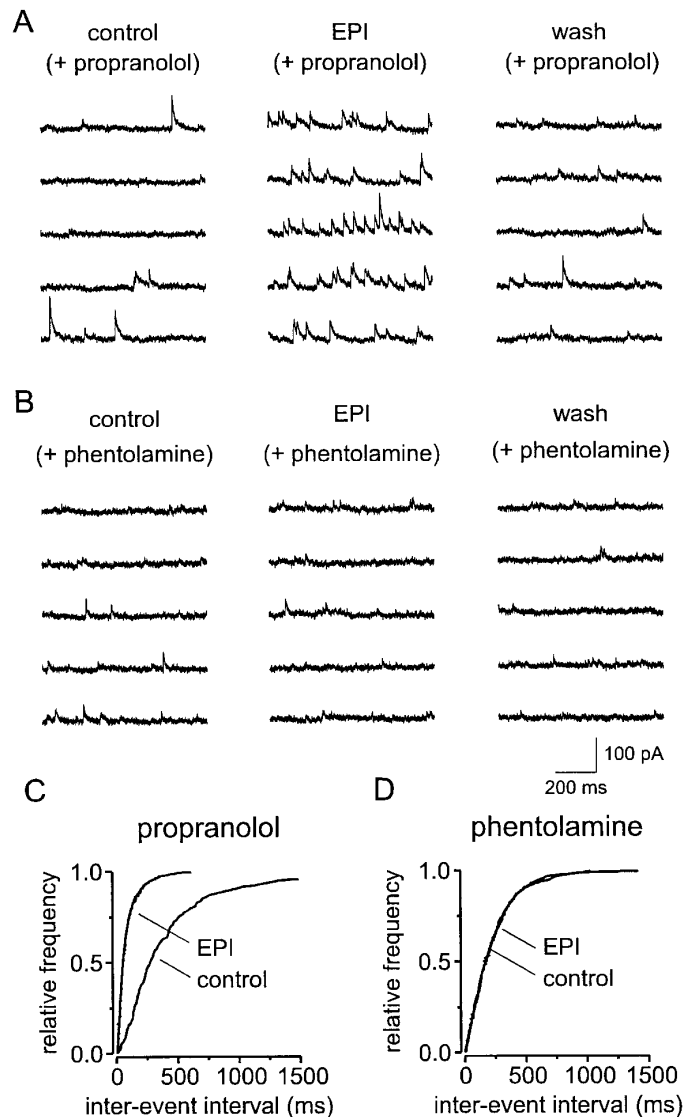


FIG. 7. EPI increases frequency of sIPSCs through activation of α -adrenoceptors. Each trace in *A* and *B* represents a continuous 4-s recording. *A*: preincubation of slice with propranolol (10 μ M) did not prevent increase in sIPSC frequency elicited by EPI (10 μ M). *B*: preincubation of a different slice with phentolamine (10 μ M) prevented EPI (10 μ M) from eliciting an increase in frequency of sIPSCs. *C*: cumulative probability plot of data from *A*, illustrating that EPI, in presence of propranolol, significantly ($P < 0.005$, K-S test) decreased interevent interval. *D*: cumulative probability plot of data from *B*, illustrating that EPI, in presence of phentolamine, did not significantly ($P > 0.005$, K-S test) decrease interevent interval.

cortex, the modulation of mIPSCs by EPI was investigated. After blockade of all action potential-dependent activity with TTX, spontaneously occurring mIPSCs could be detected (Edwards et al. 1990; Otis et al. 1991; Salin and Prince 1996). However, mIPSC frequency in neurons recorded from P9–12 slices was low and the number of events collected during 2 min samples was too small to facilitate statistical analyses of these events. Consequently, mIPSCs were only examined in detail in recordings from neurons of P15–18 slices in which the mean frequency and amplitude of mIPSCs was 15.9 ± 4.3 Hz and 24.3 ± 1.8 pA, respectively ($n = 9$). In three of nine neurons, adrenoceptor activation by EPI produced a significant ($P < 0.005$, K-S test), reversible increase in the frequency and amplitude of mIPSCs, as shown in the traces of Fig. 8A and the cumulative probability plots of Fig. 8, B and C. Alterations in the apparent frequency of mIPSCs might have arisen from the increase in their amplitude that made previously undetectable events visible above the noise. To eliminate this possibility, we examined the relationship between the holding potential and the frequency of detected events to determine whether or not increases in mIPSC amplitude produced by increases in driving force would affect mIPSC frequency (Fig. 8D). Indeed, changes in holding potential did influence the number of detected events when driving force was small. For holding potentials of 0 mV (the standard experimental situation) and more positive however, equivalent mIPSC frequencies were obtained (Fig. 8D). This suggests that under our experimental conditions changes in amplitude of mIPSCs would not influence mIPSC frequency and further that the increases in frequency of mIPSCs observed after bath application of EPI are likely to be a result of a real increase in the number of events, rather than an increase in the amplitude of previously undetected events. This conclusion was further supported by the observation that mIPSC amplitude was nearly linearly related to the command voltage (Fig. 8E), indicating that the lack of increase in the frequency of mIPSCs at more positive holding potentials (Fig. 8D) could not have been a result of an inability to detect small events.

The values for R_n in this series of experiments were in the same range as those given above for neurons in which the effect of EPI on eIPSCs was under study. As described for all other cells in this study, EPI did not produce a significant ($P > 0.1$; paired *t*-test) alteration in R_n during experiments in which mIPSCs were under scrutiny.

DISCUSSION

These data show that adrenoceptor activation can modulate the amplitude of pharmacologically isolated monosynaptic eIPSCs recorded in layer V neurons of sensorimotor cortex, in the absence of any detectable change in either passive membrane properties or the postsynaptic response to applied GABA. The effects of EPI on the amplitude of eIPSCs were heterogeneous at both ages examined but were always accompanied by a profound increase in sIPSC frequency. Examination of mIPSCs in slices from P15–18 rats revealed that EPI produced increases in the frequency and amplitude of these events in a subpopulation of neurons. These data are consistent with an adrenergic modulation of inhibition arising from activation of adrenoceptors located on inhibitory interneurons.

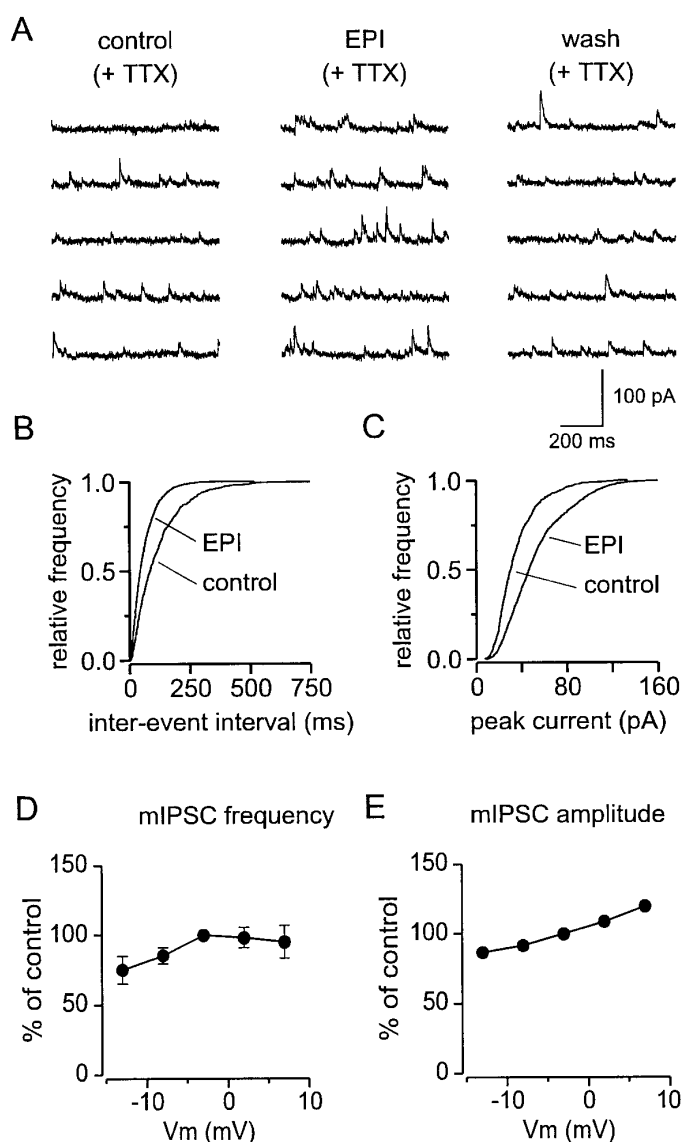


FIG. 8. EPI produces an increase in both frequency and amplitude of miniature IPSCs (mIPSCs) in a subpopulation of neurons. A: frequency of mIPSCs was increased during exposure of slice to EPI (10 μ M). Each trace represents a continuous 4-s sample. B and C: cumulative probability plots for cell of A indicate that both frequency and amplitude of mIPSCs were significantly ($P < 0.005$; K-S test) increased by EPI. D: data from 3 neurons indicate that increasing driving force for mIPSCs positive to 0 mV does not increase apparent frequency of mIPSCs. E: relationship between holding potential and normalized mean mIPSC amplitude for 3 neurons of D.

Activation of adrenoceptors affects both sIPSCs and eIPSCs

The amplitude of eIPSCs was depressed by EPI in a proportion of neurons recorded from both P9–12 and P15–18 rats. In all cases, this effect was accompanied by an increase in the frequency of sIPSCs. The observed elevation in sIPSC frequency was the result of activation of α -adrenoceptors located on presynaptic interneurons, stimulation of which underlies depolarization and action-potential discharge in hippocampal interneurons (Bergles et al. 1996). A similar α -adrenoceptor-mediated depolarization of neocortical interneurons is probably responsible for the increase in sIPSC

frequency observed in our experiments. One consequence of the increase in these spontaneous inhibitory events could be an elevation in the level of GABA in the synaptic cleft. Theoretically, this could produce activation of presynaptic GABA_B autoreceptors that would depress the amplitude of eIPSCs (Bowery et al. 1980; Davies et al. 1990; Deisz and Prince 1989; Dutar and Nicoll 1988; Harrison 1990; Thompson and Gähwiler 1989c). In recent experiments, we confirmed that such a mechanism was responsible for the EPI-induced depression of eIPSCs by demonstrating that CGP 35348, a GABA_B receptor antagonist, could block this depression without preventing the increase in sIPSC frequency (Bennett et al. 1997).

In both P9–12 and P15–18 slices, there were also cells in which adrenoceptor stimulation did not affect the amplitude of evoked events, despite increases in sIPSC frequency. Previous studies have demonstrated that there is heterogeneity in presynaptic regulation of inhibitory synapses (Ault and Nadler 1982; Lambert and Wilson 1993; Pearce et al. 1995). In the hippocampus, regulation of GABA release by GABA_B autoreceptors is only present at a subset of inhibitory synapses (Lambert and Wilson 1993). A similar arrangement in the neocortex might explain instances in which EPI did not affect the amplitude of eIPSCs. In some neurons from both P9–12 and P15–18 slices, EPI application increased the amplitude of eIPSCs. One explanation for this observation is an alteration in terminal excitability independent of GABA_B receptor activation. Support for this suggestion comes from the finding that, in slices from P15–18 rats, the frequency and amplitude of mIPSCs was elevated in about the same proportion of neurons that showed enhancement of eIPSC amplitude after adrenoceptor activation. Although there is not a well-documented connection between alterations in mIPSCs and corresponding changes in eIPSCs, this remains an attractive possibility. Comparison of the amplitude of eIPSCs with mIPSCs and sIPSCs indicates that the evoked events are likely to arise after activation of a relatively small number of presynaptic terminals. Under these conditions, heterogeneity in the complement of receptors located on the presynaptic neurons might be reflected as significant variability in the response to EPI.

Adrenoceptor-mediated modulation of GABAergic transmission is presynaptic

There are several indirect lines of evidence that indicate that the effects of EPI on eIPSC amplitude were likely mediated through presynaptic receptors. Persistent postsynaptic alterations in R_n that followed the time course of increases or decreases in eIPSC amplitude and which might have affected evoked currents, were not detected after EPI application. This is perhaps not surprising in light of the fact that many of the known effects of adrenoceptors are produced by modulation of K⁺ currents (see Nicoll et al. 1990), which were blocked in all recorded neurons by the presence of Cs⁺ and QX-314 in the recording pipette (Adelman and French 1978; Hagiwara et al. 1976; Nathan et al. 1990). The possibility that the conductance of remote portions of dendritic membrane not adequately clamped or perfused by Cs⁺ were affected by EPI, causing alterations in eIPSCs generated distally, cannot be eliminated. However, the time course of the

eIPSC did not appear to be affected by EPI. This suggests that alterations in postsynaptic dendritic conductance in “un-clamped” areas are unlikely to be responsible for the EPI-induced changes in eIPSCs. The increased frequency of sIPSCs did not affect measurements of R_n because care was taken to measure instantaneous R_n from segments of current traces that were not contaminated by sIPSCs.

Another possible postsynaptic mechanism for EPI actions, which would not be detected by measurement of R_n , is an alteration in the postsynaptic responsiveness to GABA that could lead to alterations in eIPSC amplitude. EPI application did not however affect postsynaptic responses to iontophoretically applied GABA, making this explanation unlikely, unless junctional and nonjunctional GABA_A receptors are differently affected by EPI. The stability of GABA responses at a time when eIPSCs were affected by EPI also argues against the possibility that reductions in eIPSC amplitude resulted from a chloride accumulation in the postsynaptic cell and a consequent shift in the equilibrium potential for IPSCs (Huguenard and Alger 1986; McCarren and Alger 1985; Thompson and Gähwiler 1989a,b). Furthermore, the kinetics of eIPSCs were unaltered by EPI application irrespective of whether the amplitude of evoked events was increased or decreased, indicating that synaptically activated GABA_A receptors were unaltered after activation of adrenoceptors. Increases in sIPSC and mIPSC frequency after EPI application provide further evidence for presynaptic effects. Stimulation of adrenoceptors alters the frequency of mIPSCs in the cerebellum (Llano and Gerschenfeld 1993) and hippocampus (Gereau and Conn 1994). Changes in mIPSCs frequency presumably indicate a direct action on presynaptic terminals, whereas changes in mIPSC amplitude have been interpreted as resulting from postsynaptic effects (Thompson et al. 1993; but see Van der Kloot 1991). Although we observed concomitant changes in both mIPSC frequency and amplitude in a subpopulation of neurons, our interpretation is that both of these effects are probably presynaptic because R_n and responses to iontophoretic GABA applications were unaltered by EPI perfusion. A possible presynaptic mechanism for the increase in mIPSC amplitude is that previously silent terminals that possess a larger quantal content of GABA become activated by EPI. Alternatively, EPI might preferentially increase release at terminals that give rise to mIPSCs that are larger than the mean mIPSC amplitude.

Adrenoceptor activation produces heterogeneous effects on eIPSC amplitude

In the present study, EPI application produced depression, no effect or enhancement of eIPSCs in neurons from both P9–12 and P15–18 slices, even though sIPSC frequency was increased in all cells from both groups. Interestingly, large increases in sIPSP frequency in hippocampal CA1 pyramidal neurons elicited by activation of α -adrenoceptors (Bergles et al. 1996; Doze et al. 1991; Madison and Nicoll 1988) are not accompanied by reductions in eIPSP amplitude (Doze et al. 1991).

The difference in the response of eIPSCs to EPI application might be related to preferential activation of different receptor subtypes. Heterogeneous effects of adrenoceptor activation on the amplitude of evoked excitatory synaptic

events were described previously (Dodt et al. 1991; Gereau and Conn 1994; Scanziani et al. 1993). The difference in time course of enhancement versus depression of eIPSCs (cf. Fig. 3, A and B) would appear to be consistent with underlying differences in either the adrenoceptor subtype or the intermediary cellular mechanisms that lead to the response. The utilization of receptor-selective compounds revealed that these differences are likely the result of activation of different types of adrenoceptors. Activation of α -adrenoceptors reduces the amplitude of evoked excitatory postsynaptic potentials (eEPSPs) in the neocortex (Dodt et al. 1991) and hippocampus (Scanziani et al. 1993), while selective activation of β -adrenoceptors enhances eEPSC amplitude in hippocampal CA1 neurons (Gereau and Conn 1994). Another possibility, along the same line, is that electrical stimulation activates different anatomic subclasses of GABAergic interneurons (Houser et al. 1983; Jones 1993; Kawaguchi 1995) that possess different relative densities of α - and β -adrenoceptors. Unfortunately, only single applications of EPI could be used in the present study because of a very large desensitization as well as run down in the response of eIPSCs. These factors prevented characterization of the receptor subtype that was responsible for the modulation of eIPSC amplitude. It is clear from the results of these experiments, however, that the reduction in eIPSC amplitude in P9–12 slices is the result of a α -adrenoceptor-mediated increase of GABA release (Figs. 6 and 7) that activates GABA_B receptors located on GABAergic interneurons (Bennett et al. 1997). Therefore a final potential explanation for variability in the attenuation of eIPSCs by EPI might be differences in the distribution and density of GABA_B autoreceptors (Turgeon and Albin 1994).

In conclusion, activation of adrenoceptors located on presynaptic inhibitory interneurons produces a heterogeneous modulation of the amplitude of monosynaptic eIPSCs. However perfusion of EPI consistently increases the frequency of sIPSCs through activation of presynaptic α -adrenoceptors. Irrespective of the effect on eIPSCs, activation of adrenoceptors could, therefore, result in increased tonic shunting inhibition. These data indicate that presynaptic adrenoceptors are involved in regulation of inhibition in the sensorimotor region of the neocortex.

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