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Influence of hypoxia on excitation and GABAergic inhibition in mature and developing rat neocortex

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Abstract. To analyze the functional consequences of hypoxia on the efficacy of intracortical inhibitory mechanisms mediated by gamma-aminobutyric acid (GABA), extra- and intracellular recordings were obtained from rat primary somatosensory cortex in vitro. Hypoxia, induced by transient N_2 aeration, caused a decrease in stimulus-evoked inhibitory postsynaptic potentials (IPSPs), followed by a pronounced anoxic depolarization. Upon reoxygenation, the fast (f-) and long-latency (l-) IPSP showed a positive shift in the reversal potential by 24.4 and 14.9 mV, respectively. The peak conductance of the f- and l-IPSP was reversibly reduced in the postanoxic period by 72% and 94%, respectively. Extracellular field potential recordings and application of a paired-pulse inhibition protocol confirmed the enhanced sensitivity of inhibitory synaptic transmission for transient oxygen deprivation. Intracellular recordings from morphologically or electrophysiologically identified interneurons did not reveal any enhanced susceptibility for hypoxia as compared to pyramidal cells, suggesting that inhibitory neurons are not selectively impaired in their functional properties. Intracellularly recorded spontaneous IPSPs were transiently augmented in the postanoxic period, indicating that presynaptic GABA release was not suppressed. Developmental studies in adult (older than postnatal day 28), juvenile (P14-18), and young (P5-8) neocortical slices revealed a prominent functional resistance of immature tissue for hypoxia. In comparison with adult cortex, the hypoxia-induced reduction in excitatory and inhibitory synaptic transmission was significantly smaller in immature cortex. Our data indicate a hypoxia-induced distinct reduction of postsynaptic GABAergic mechanisms, leading to the manifestation of intracortical hyperexcitability as a possible functional consequence.

Key words: Hypoxia – Neocortical slice – Synaptic transmission – GABAergic inhibition – Interneurons – Development – Rat

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Introduction

Cerebral ischemia, caused by cardiac arrest or selective occlusion of cerebral arteries, may lead to chronic neurologic disorders (Adams 1975; Cocito et al. 1982). Clinical studies (Calame et al. 1985; Karch 1982) and different experimental models (Ikonomidou et al. 1989; Johnston et al. 1991; Silverstein et al. 1987; Slotkin et al. 1986) have shown that severe consequences for neuronal organization can result from perinatal hypoxia. Epilepsy is one of the long-term functional deficits resulting from oxygen deprivation during critical periods of early development (Towbin 1970). Epileptogenesis is often related to a reduction in the efficacy of GABAergic inhibition. Indeed, selective loss of inhibitory interneurons (Esclapez and Trottier 1989; Sloper et al. 1980; Sloviter 1987; Takahashi et al. 1991), a reduction of GABAergic synaptic terminals (Houser et al. 1986) and a decrease in the GABA content in the cerebrospinal fluid (Löscher et al. 1981; Manyam et al. 1980) have been observed in human epileptic tissue and different animal models of epilepsy (for review, see Ribak 1991). However, this so-called GABA hypothesis could not be confirmed in other model systems (Mody et al. 1987; Perreault and Avoli 1991). Even with hypoxia, different effects on GABAergic cells have been reported (Nitsch et al. 1989; Romijn et al. 1988, 1992; Tecoma and Choi 1989; for review, see Meyer 1989; Schmidt-Kastner and Freund 1991). These conflicting results may point to regional variations in hypoxia sensitivity of GABAergic systems but also indicate a pronounced variation in the effect of ischemia on the GABAergic system in different model systems (e.g., in vivo vs cell culture). More subtle modifications that are difficult to detect with morphological techniques may contribute to these discrepancies. They would be unravelled by electrophysiological and molecular biological studies of the efficacy of GABAergic transmission. Such changes may occur at the subcellular level, such as, e.g., the GABA receptor complex (Mileson et al. 1992).

In a previous in vitro study we demonstrated with intra- and extracellular recording techniques an in-

creased sensitivity of the inhibitory synaptic transmission to transient, short-term hypoxia in adult rat neocortex (Luhmann and Heinemann 1992b). These observations have been recently confirmed by Rosen and Morris (1993) in supragranular pyramidal neurons from mature rat neocortical slices. In order to further elucidate the functional impact of an ischemic insult on GABAergic inhibition, we investigated in more detail the effect of long-term hypoxia on GABAergic synaptic mechanisms in the neocortical slice preparation. We were especially interested in the effects of oxygen deprivation on spontaneous and stimulus-evoked inhibitory postsynaptic potentials (IPSPs) and in the response of interneurons to hypoxia. In addition, we investigated possible age-dependent alterations in the susceptibility of the neocortex to hypoxia by analysing extracellularly recorded field potentials (FPs) in slices obtained from rats of different developmental stages. A resistance of immature tissue to oxygen deprivation has been previously reported by Hansen (1977) and Cherubini et al. (1989; for review, see Hansen 1985). Some of these results have been presented in abstract form (Luhmann and Heinemann 1992a).

Materials and methods

The preparation and maintenance of rat neocortical slices in vitro were similar to those described previously (Luhmann and Heinemann 1992b). In brief, young (P5-8), juvenile (P14-18), and adult (>P28) Wistar rats were deeply anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg bodyweight), decapitated, and a small block of the brain including the primary somatosensory cortex (Zilles and Wree 1985) was rapidly removed and placed in cold (4° C), oxygenated artificial cerebrospinal fluid (ACSF). Coronal slices (400–500 µm) were cut on a Dosaka vibratome and transferred to an interface-type recording chamber or to an incubation-storage chamber. The control bathing solution (ACSF) contained: NaCl 124 mM, KCl 3 mM, NaH_2PO_4 1.25 mM, $MgSO_4$ 1.8 mM, $CaCl_2$ 1.6 mM, $NaHCO_3$ 26 mM, and glucose 10, and when saturated with 95% O_2/5% CO_2 the ACSF had a pH of 7.4 at the experimental temperature of 34–35 °C. Hypoxia was induced by changing the mixture of the aerating gas in the interface-type recording chamber from 95% O_2/5% CO_2 to 95% N_2/5% CO_2. Two experimental protocols were used to evaluate hypoxia-induced functional alterations. First, in intracellular recordings hypoxia was generally terminated by reoxygenation immediately after occurrence of the rapid voltage deflection initiating the anoxic depolarization (AD). Second, in extracellular recordings and for comparison of the effects in slices from different age groups the duration of hypoxia was always kept constant at 2.5 min.

Extracellular FPs were recorded with ACSF-filled electrodes (resistance 2–5 MΩ). Synaptic responses in layers II/III were obtained by electrical stimulation (200 µs duration) of the underlying white matter/layer VI with a bipolar tungsten electrode. If not otherwise stated, stimulus strength was adjusted to twice the intensity needed to evoke a maximal FP response or an action potential. Only slices in which orthodromic stimulation evoked a FP response of more than 1 mV were chosen for further electrophysiological investigations. To minimize a participation of antidromic activity, only FP responses with a stimulus-to-peak latency of more than 3 ms were included in the data analysis. A paired-pulse inhibition protocol was used to calculate the efficacy of intracortical GABAergic inhibition from the following formula (for details, see Luhmann and Heinemann 1992b): percentage inhibition = $[(FP_1 - FP_2)/FP_1] \times 100$ where FP_1 is peak-to-peak FP response to first stimulus; FP_2, FP response to second stimulus. The strength of inhibition was strongly dependent on the interstimulus interval (see Fig. 1 in Luhmann and Heinemann 1992b) and on the stimulus intensity (Fig. 1). Very low stimulus intensities elicited a FP response that amounted to only 10–30% of the maximal response and often resulted in paired-pulse facilitation instead of inhibition (Fig. 1B). However, when the amplitude of the FP response was larger than 30% of the maximal response, the response to the second stimulus was always suppressed. Strong paired-pulse inhibition could be demonstrated with interstimulus intervals between 20 and30 ms in neocortical slices obtained from adult and juvenile rats (see Figs. 9A and 10A, respectively). In agreement with previous intracellular observations (Kriegstein et al. 1987; Luhmann and Prince 1991), cortices from young animals did not reveal any intracortical inhibitory mechanisms and prominent paired-pulse suppression could not be detected (see Fig. 11B, trace 1). For that reason, only excitatory synaptic responses were analyzed in this age group.

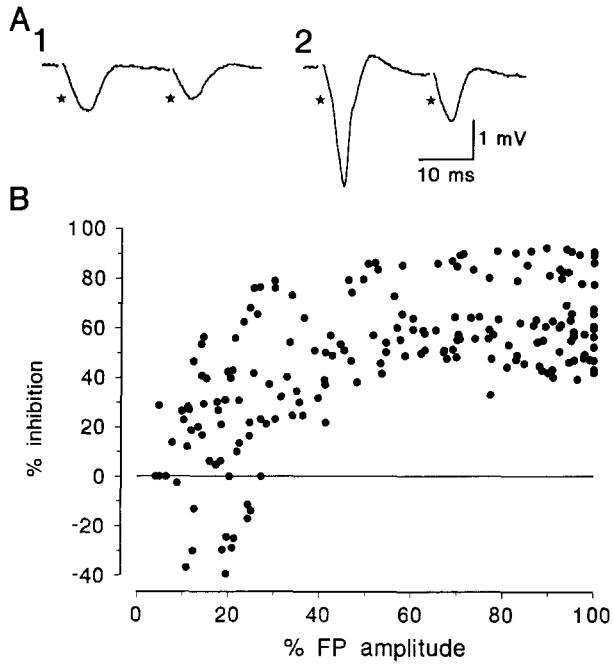

Fig. 1A, B. Relationship between relative amplitude of excitatory field potential (*FP*) response (*x*-axis) and strength of paired-pulse inhibition (*y*-axis) in layers II/III of adult rat neocortical slices. **A** Characteristic FP response to a double stimulus with a constant interstimulus interval of 20 ms at low (1) and high (2) stimulus intensity. The FP response to the first stimulus in (1) amounted to 34% of the maximal response (100%) shown in 2. **B** Scatter diagram showing the relationship between the percentage of the FP amplitude (100% = maximal excitatory FP response amplitude to the first stimulus) and percentage of paired-pulse inhibition (for details see Materials and Methods, $n = 193$ measurements in 13 FPs)

Intracellular recordings were performed with microelectrodes (1.5 mm OD, 0.86 mm ID; resistance 60–120 MΩ) pulled with a horizontal air-jet puller (P-87; Sutter Instrument) and backfilled with 2 M potassium acetate (ph 7.4) containing 1–2% biocytin (Horikawa and Armstrong 1988). Only cells with stable resting membrane potentials more negative than −65 mV and with an input resistance of more than 20 MΩ were included in the data analysis. Neurons were classified into regular-spiking and fast-spiking cells, according to the functional criteria established by McCormick et al. (1985). Intracellular electrodes were connected to a high-impedance amplifier (Neuro Data) with a bridge circuit for simultaneous current injection and voltage measurement. A storage oscilloscope and a chart recorder (Astromed) were used to display the recorded signals during the experiment. Signals were recorded on-line and analyzed with the TIDA software program (Batelle Institute, Frankfurt, Germany). Stimulus-evoked IPSPs in supragranular neurons were elicited by electrical stimulation of the un-

derlying white matter/layer VI. The stimulus intensity was adjusted to twice the threshold value necessary to evoke an action potential at resting membrane potential. The reversal potential of the IPSPs (E_{IPSP}) was calculated by linear regression from a plot of the peak response amplitude versus membrane potential (see also Fig. 5G). Peak conductances (G_{IPSP}) were determined by linear regression analysis from the slope of the plot of the relation between the membrane potential deflection at the IPSP peak versus injected current (Connors et al. 1988; Luhmann and Prince 1991; see also Fig. 5H). To avoid any contamination of voltage-activated conductances this analysis was performed in the linear range of the membrane's current-voltage relationship. The pure IPSP conductance was determined by subtracting the cell's resting conductance from the estimated conductance at the IPSP peak amplitude. In experiments designed to analyze the effects of hypoxia on spontaneous IPSPs, the electrodes were filled with 2 M potassium nitrate (pH 7.4). Diffusion of nitrate into the cell induces a positive shift in the reversal potential of miniature chloride-dependent spontaneous IPSPs. In contrast to chloride ions, nitrate ions are transported less efficiently out of the cell via active transport mechanisms and depolarizing spontaneous IPSPs can be reliably recorded over long time periods (Misgeld et al. 1986; Thompson et al. 1988, 1989). The blockade of spontaneous IPSPs by bicuculline indicates that these events are $GABA_A$ receptor-mediated and chloride-dependent (Luhmann and Prince 1991). For quantitative analysis of depolarizing spontaneous IPSPs the integral above the resting membrane potential was measured. To compare the functional modifications during different phases of hypoxia, spontaneous and stimulus-evoked IPSPs were analyzed under control conditions, shortly before AD onset (pre AD), after termination of AD (post AD), and during the recovery phase (see Fig. 4B, 2). For statistical analysis a Student's *t*-test was performed on the data. If not otherwise noted, data throughout this report are given as mean ± SD.

The drugs *DL*-amino-phosphonovaleric acid (APV, 150–300 µM; Sigma), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 50–100 µM; Tocris Neuramin), and 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(*F*)quinoxaline (NBQX, 50–100 µM; Novo Nordisk) were applied locally via a broken micropipette to the slice surface in order to block excitatory synaptic transmission.

For later morphological studies, neurons were labelled intracellularly with biocytin by injecting depolarizing current pulses into the cell. Slices were fixed in 4% paraformaldehyde for more than 24 h and washed in 0.1 M phosphate buffer. After cryoprotection of the tissue with 30% sucrose, slices were resectioned at 60–120 µm on a freezing microtome. Sections were incubated in avidin-biotin-peroxidase complex (ABC) reagent (Vector) and subsequently reacted with diaminobenzidine (for details, see Tseng et al. 1991). The morphology and spiny or nonspiny appearance of single-labelled cells was investigated with a Leitz light microscope. Morphometrical two-dimensional measurements on single cells were performed with a digitizing tablet and a graphic program (Sigma-Scan; Jandel Scientific).

Results

Data pool

Intracellular recordings were obtained from 94 neurons in coronal slices from the primary somatosensory cortex of the adult rat. On the basis of their membrane properties and their response pattern to injection of suprathreshold depolarizing current pulses, 89 neurons located in layers II/III were classified as regular-spiking cells (McCormick et al. 1985). In addition, 5 neurons with fast-spiking characteristics, presumed to be interneurons, were recorded in supragranular layers and in layer V. A detailed morphological analysis of intracellularly biocytin-filled cells was performed on 33 supragranular cells (Schröder and Luhmann 1992). Out of this sample, 29 neurons had the characteristic morphology of upper-layer pyramidal cells, with a complex, spiny dendritic tree. The remaining 4 neurons showed a less extensive, nonspiny dendritic arborization (Fig. 2). In addition, the mean soma area of these cells (133.4 ± 22 µm^2, $n=4$) was significantly ($P<0.001$) smaller than that of the spiny pyramidal neurons in the same laminae (279.8 ± 153.1 µm^2, $n=18$). The 5 fast-spiking cells and the 4 nonspiny neurons displayed action potentials of relatively short duration (0.51 ± 0.18 ms at half maximal amplitude, $n=9$), no or weak spike frequency adaptation, and monophasic afterhyperpolarizations (Figs. 3, 6A). All these morphological and electrophysiological properties are characteristic of neocortical inhibitory interneurons. We therefore classified these 9 cells as local interneurons. The investigated regular- and fast-spiking cells displayed a mean resting membrane potential (V_m) of -78.8 ± 5.2 mV, a spike amplitude of 103.5 ± 6.0 mV, and an input resistance (R_N) of 33.9 ± 10.2 MΩ. Spontaneous IPSPs were recorded and analyzed in 21 regular spiking cells.

Extracellular FP responses to hypoxia were studied in 17 adult, 12 juvenile, and 10 young neocortical slices. Control recordings under permanent oxygen supply were obtained from another 6 adult, 4 juvenile, and 6 young slices. In FP recordings in young cortical slices, excitatory synaptic transmission was blocked by the *N*-methyl-D-aspartate (NMDA) and non-NMDA receptor antagonists APV and CNQX or NBQX, respectively, to estimate the role of antidromic activity in these responses. The mean stimulus-to-peak latency of the FP responses in these age groups was 6.5 ± 1.5 ms (23 adult FPs), 6.4 ± 1.8 ms (15 juvenile FPs), and 5.2 ± 3.3 ms (20 young FPs). Some of the FP recordings and intracellular data obtained from adult animals were included in a previous report (Luhmann and Heinemann 1992b).

Effects of long-term hypoxia on intrinsic membrane properties

The effects of short-term hypoxic episodes (2–4 min) on neocortical function in vitro have been previously reported by us (Luhmann and Heinemann 1992b) and will be described only in brief. Extracellular recordings during hypoxia revealed only minor deflections in the d.c. potential (Fig. 4A, row 1). The initial intracellular response consisted in 62% of the cells tested ($n=74$) of a membrane hyperpolarization (Figs. 4A, row 2, 8B) and in the remaining 38% of a gradual depolarization (Fig. 8A). Both response types were characterized by a prominent decrease in neuronal input resistance (Fig. 4A, row 2). In spite of different responses, a contribution of ATP-sensitive K^+ conductances has been demonstrated in neocortical neurons, both to the initial hyperpolarization and to the initial depolarization (see Discussion). This initial response was generally followed by a postanoxic hyperpolarization (Fig. 4A, row 2) with a more negative E_{hyp} (Luhmann and Heinemann 1992b). Prolongation of hy-

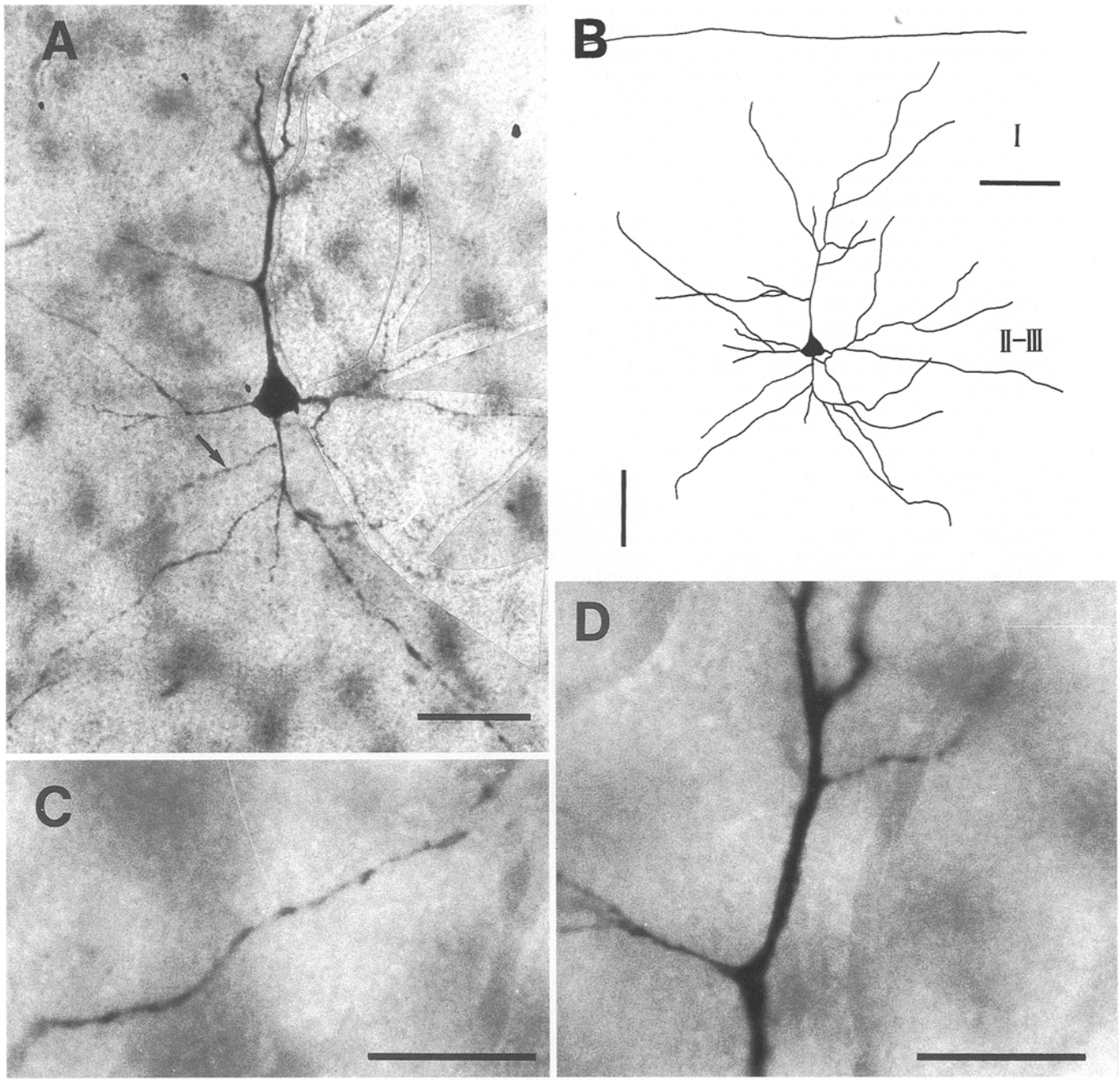

Fig. 2A–D. Morphology of intracellularly biocytin-labelled non-spiny neuron in layer III of adult rat primary somatosensory cortex. Photomontage (**A**) and camera lucida drawing (**B**) of the cell; photograph of proximal (**C**) and apical (**D**) dendrite at higher magnification. *Arrow* in **A** points to dendrite shown in **C**. Unspecific background due to Nissl staining. *Calibration bars* 30 μm (**A**), 50 μm (**B**), 20 μm (**C**, **D**)

poxia caused a pronounced AD, which could be recorded extracellularly as a sudden negative deflection in the d.c. potential by up to 24.6 mV (Fig. 4B, row 1) and a corresponding intracellularly recorded transmembranal depolarization ($n=27$ cells) by 55.4 ± 10.6 mV (Fig. 4B, row 2). The onset latency of the intracellularly recorded AD was 3.5 ± 1.5 min and the AD duration at half maximal amplitude was 1.5 ± 0.5 min. The AD was associated with a significant decrease in input resistance (see voltage responses to hyperpolarizing current pulses in Fig. 4B, row 2) and complete loss of synaptic transmission. Upon reoxygenation the membrane gradually repolarized and input resistance and synaptic function recovered (Fig. 4B). A post-AD depolarization of 4.0 ± 1.7 mV occurred in 7% of the neurons. However, the majority of the cells showed a gradual repolarization to a stable V_m associated with an increase in spontaneous activity during the recovery phase.

Stimulus-evoked IPSPs

Hypoxia-induced modifications in the E_{IPSP} and the G_{IPSP} of the fast, chloride-dependent IPSP (f-IPSP) and long-lasting, potassium-dependent IPSP (l-IPSP) were analyzed in nine supragranular, regular spiking cells (Fig. 5). Under control conditions (Fig. 5A,B), suprathreshold synaptic stimulation evoked a f-IPSP with $E_{f\text{-}IPSP} = -75.3 \pm 4.5$ mV (open circles in Fig. 5G) and $G_{f\text{-}IPSP} = 139.6 \pm 61.2$ nS (open circles in Fig. 5H), followed by a l-IPSP with $E_{l\text{-}IPSP} = -88.9 \pm 4.0$ mV (filled squares in Fig. 5G) and $G_{l\text{-}IPSP} = 28.0 \pm 10.2$ nS (filled squares in Fig. 5H; control in Fig. 5I,J). During the anoxic hyperpolarization (Fig. 5C) the f- and l-IPSP showed a significant ($P < 0.02$) depolarizing shift in their E_{IPSP} to -66.0 ± 9.8 mV and -80.2 ± 7.3 mV, respectively (pre AD in Fig. 5I). Although G_{IPSP} of the two IPSPs was reduced to 92.2 ± 60.9 nS and 14.9 ± 15.9 nS, respectively, this effect was not significant at the $P < 0.05$ level (pre AD in Fig. 5J). A pronounced effect on both IPSPs could be observed after termination of the AD (Fig. 5D,E). The f- and l-IPSP displayed a significant ($P < 0.001$) depolarizing shift in E_{IPSP} to -50.9 ± 5.9 mV and -74.0 ± 5.9 mV, respectively, associated with a significant ($P < 0.001$) decline in G_{IPSP} to 38.9 ± 23.1 nS and 1.4 ± 2.4 nS, respectively (post AD in Fig. 5I,J). These effects were only transiently expressed and full recovery in the two parameters could be observed for both IPSPs (Fig. 5F,I,J).

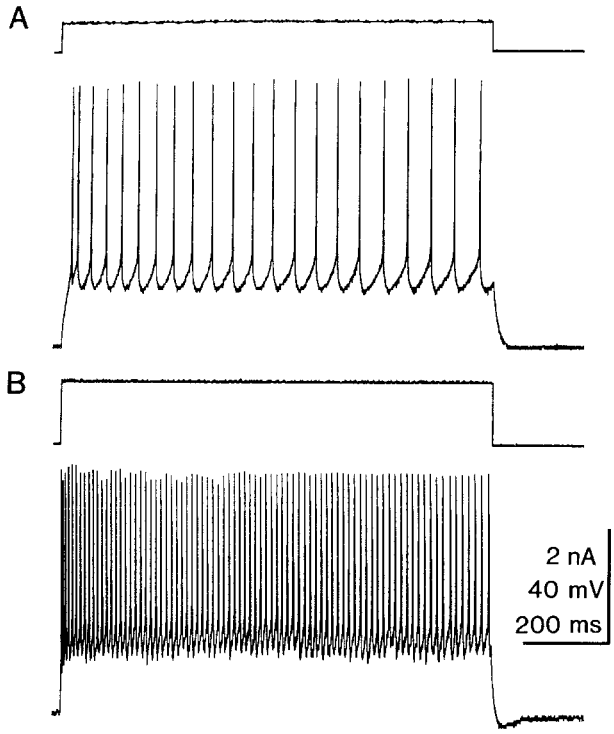

Fig. 3. Repetitive discharge pattern in response to injection of suprathreshold depolarizing current pulse of 1 s duration in a non-spiny neuron (**A**) and fast-spiking cell (**B**). Recording in **A** was obtained from the cell shown in Fig. 2

Response of inhibitory interneurons

The effect of hypoxia on presumably GABAergic cells was studied in 5 electrophysiologically (fast-spiking characteristics; Figs. 3B, 6A) and 4 morphologically (non-spiny dendrites and small somata; Fig. 2) identified interneurons with short-duration action potentials. These interneurons revealed neither an increased sensitivity nor any other significant difference in their responsiveness to

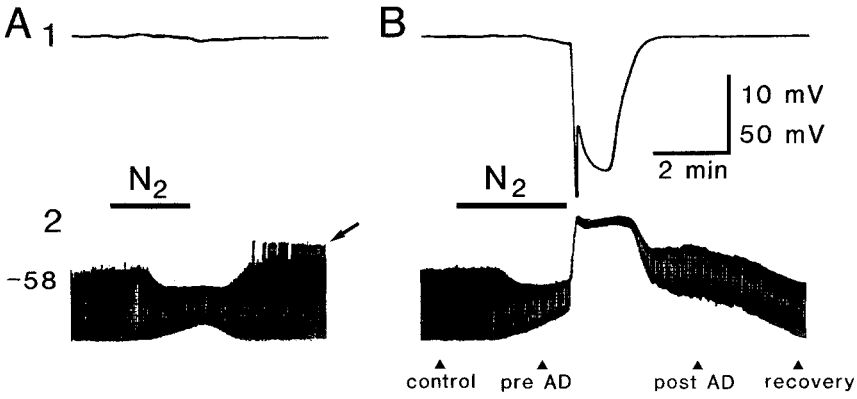

Fig. 4. Extra- (*row* 1) and intracellular (*row* 2) response to 2 min (**A**) and 2.8 min (**B**) hypoxia recorded simultaneously in supragranular layers of adult rat primary somatosensory cortex in vitro. Interval of nitrogen (N_2) aeration is indicated by *horizontal bar*. Membrane potential of intracellularly recorded cell was depolarized to -58 mV by positive current injection. Hyperpolarizing current pulses of 300 ms duration were injected at 0.5 Hz into the cell to measure hypoxia-induced changes in membrane input resistance (current trace not shown). Owing to high-frequency injection of current pulses, voltage responses in *row* 2 appear as *filled area*. **A** Hypoxia of 2 min duration induces only minor deflections in extracellularly recorded d.c. potential (1). Intracellular response to hypoxia consists of a membrane hyperpolarization associated with a prominent decrease in input resistance (2). Upon reoxygenation, hyperpolarizing current pulses evoke action potentials due to an increase in anodal break response (*arrow*). Action potentials are truncated. **B** Same cell and protocol as in **A**, but duration of hypoxia was increased to 2.8 min. Extracellular response consists of a rapid negative shift in the d.c. potential by 20 mV (1). The corresponding intracellular response is a sudden anoxic depolarization (*AD*) of 43 mV with pronounced reduction in input resistance (2). Upon reoxygenation, extra- and intracellularly recorded signals gradually recover to control values. *Labels below trace* (*row* 2) indicate periods in which data were analyzed. Negativity in field potential recordings is *downward*

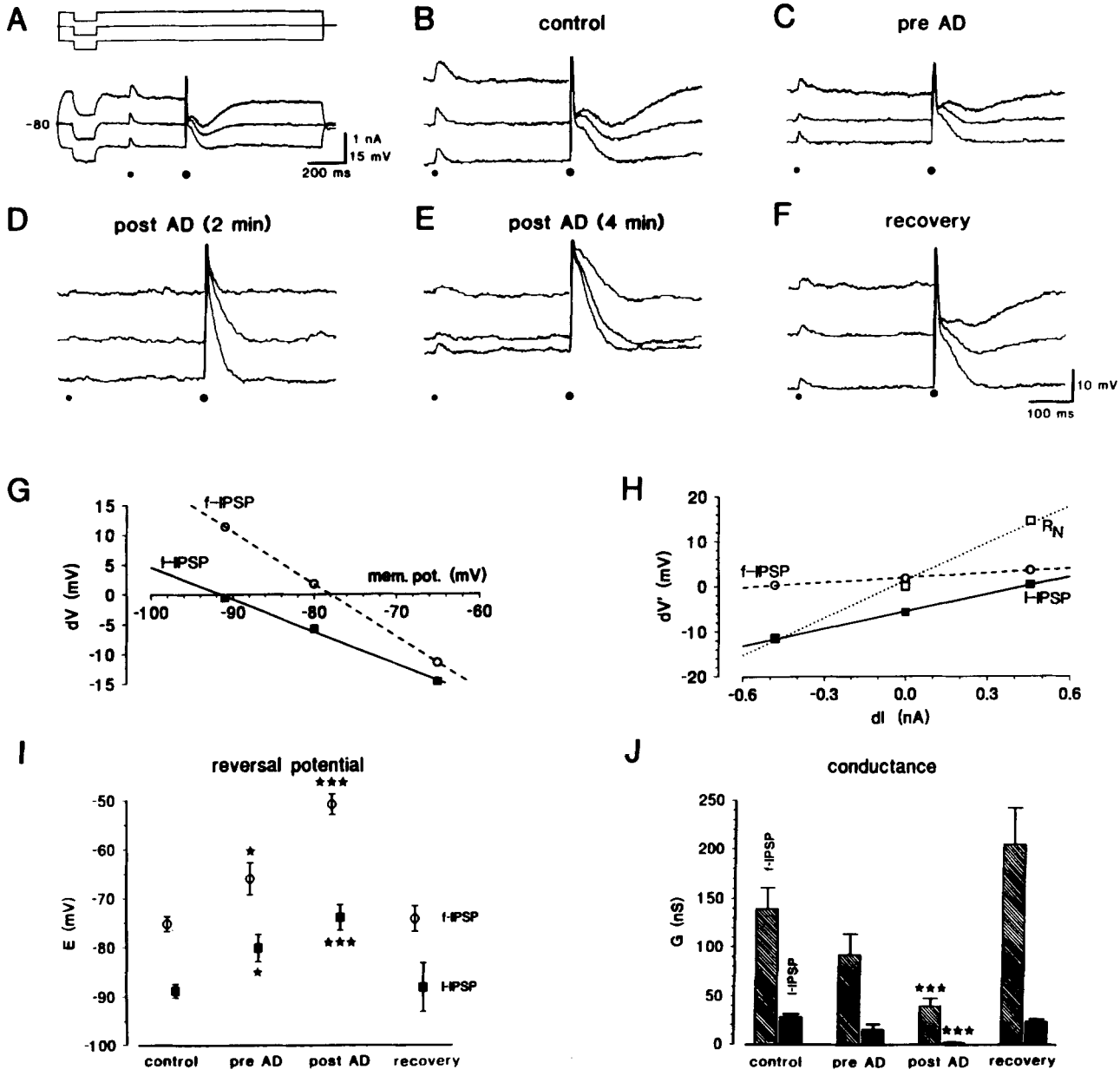

Fig. 5A-J. Effect of hypoxia on stimulus-evoked inhibitory postsynaptic potentials (IPSPs). **A** current protocol (*upper trace*) and corresponding voltage response (*lower trace*) with orthodromically evoked excitatory postsynaptic potential (EPSP; *small dot*) and biphasic IPSP (*large dot*). EPSPs and IPSPs were elicited by electrical stimulation of the underlying white matter/layer VI via the same stimulating electrode. Low, subthreshold stimulus intensities were adjusted to evoke a pure EPSP. To elicit a biphasic IPSP, the stimulus intensity was increased to twice the threshold value necessary to evoke an action potential at resting membrane potential. Resting membrane potential $V_m = -80$ mV is indicated *left* of the voltage trace. **B-F** Same cell and protocol as in **A**, but at different times and on different voltage scales. Responses were obtained: **B** under control conditions; **C** before the anoxic depolarization (*pre AD*); **D** 2 min and **E** 4 min after the anoxic depolarization (*post AD*); and **F** during the recovery period. Note suppression of IPSPs in **C-D** and long-lasting depolarizing postsynaptic potential in **E**. **G** Calculation of the reversal potential of the fast IPSP (*f-IPSP*; *open circles, dashed line*) and long-lasting IPSP (*l-IPSP*; *filled squares, solid line*) by linear regression analysis. **H** Calculation of the neuronal input resistance (R_N; *open squares, dotted line*) and the membrane resistance at the peak of the f-IPSP (*open circles, dashed line*) and l-IPSP (*filled squares, solid line*) by linear regression. Data in **G** and **H** were obtained from the cell under control conditions (**A, B**). **I** Hypoxia-induced modifications in the mean reversal potential of the f-IPSP (*open circles*, $n = 9$) and l-IPSP (*filled squares*, $n = 9$). *Bars on each symbol* indicate SEM. **J** Changes in mean peak conductance of the f-IPSP (*shaded bars*, $n = 8$) and l-IPSP (*filled bars*, $n = 8$). *Bars on each column* symbolize SEM. *Asterisks* in **I** and **J** represent significant differences: $^*P < 0.02$; $^{***}P < 0.001$ (Student's *t*-test)

hypoxia as compared to regular spiking pyramidal cells. The initial response to long-term hypoxia in 6 of the 9 interneurons tested was an anoxic hyperpolarization (arrow in Fig. 6B, trace 1), with $E_{hyp} = -83.4 \pm 6.3$ mV ($n = 5$). In the 3 remaining cells, hypoxia caused a membrane depolarization, with $E_{dep} = -68.4 \pm 1.6$ mV ($n = 2$). As in regular spiking spiny pyramidal cells (see above), the response to long-term hypoxia was a prominent AD (Fig. 6B) with a mean onset latency of 3.5 ± 0.6 min ($n = 6$), amplitude of 53.3 ± 11 mV ($n = 7$), and duration at

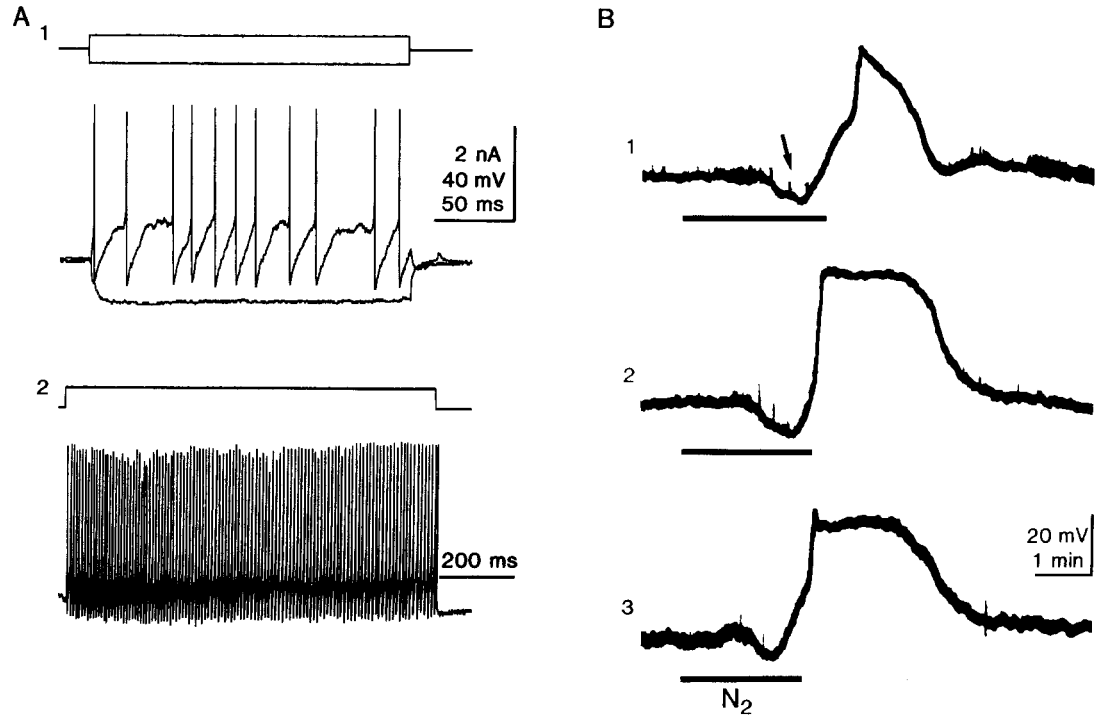

Fig. 6A, B. Response of a presumed interneuron with fast-spiking characteristics to hypoxia. **A** Suprathreshold current injection (*upper row*) elicits short spikes with typical afterhyperpolarizations (*lower row*) (1). Injection of depolarizing current pulse of 1 s duration evokes high-frequency discharge. Note lack of spike frequency adaptation (2). **B** Response of the cell shown in **A** to three subsequent episodes of hypoxia of 2.5 (1), 2.3 (2), and 2.1 min (3) duration (*horizontal bars below voltage traces*). Recovery interval between hypoxic periods was 10.5 and 13.5 min. Note initial hyperpolarization (*arrow* in B, trace 1) before pronounced anoxic depolarization. Resting membrane potential and input resistance of this cell: $V_m = -69$ mV and $R_N = 60$ MΩ, respectively

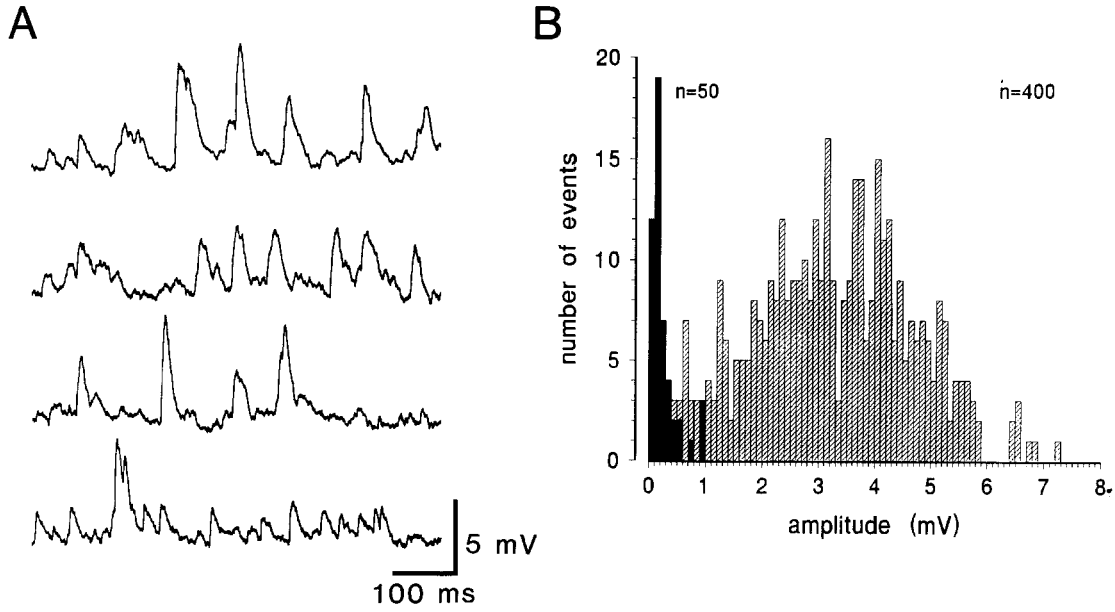

Fig. 7A, B. Depolarizing spontaneous inhibitory postsynaptic potentials (IPSPs) recorded in supragranular regular spiking cells with electrodes containing 2 M potassium nitrate. **A** Example of spontaneous IPSPs recorded in one cell in normal artificial cerebrospinal fluid (ACSF) at a resting membrane potential V_m of –79 mV. **B** Amplitude histogram of spontaneous IPSPs (*shaded bars*, $n = 400$) measured in four cells. For comparison, the amplitude distribution of internal noise (*filled bars*, $n = 50$) recorded in one cell with a 2 M potassium acetate electrode is shown

half-maximal amplitude of 1.7 ± 0.4 min ($n = 4$). All interneurons tested showed complete recovery in the investigated parameters within 10–30 min following reoxygenation. The fast spiking cell shown in Fig. 6 could be even exposed to three subsequent hypoxic episodes without pronounced deleterious functional effects. The decrease in the AD onset latency and changes in the AD duration observed in this cell have been previously described in extracellularly recorded d.c. potentials following repetitive hypoxia (Kral et al. 1993).

Spontaneous IPSPs and effects of NMDA and non-NMDA antagonists on AD

Depolarizing, bicuculline-sensitive spontaneous IPSPs were recorded in 21 neurons with electrodes containing 2 M potassium nitrate (Figs. 7A, 8C, trace 1). After impalement, spontaneous IPSPs increased in frequency and amplitude, and measurements were started when spontaneous activity reached an apparent steady state. Spontaneous IPSPs varied in amplitude from 1 to 8 mV and were clearly distinguishable from equipment internal noise (Fig. 7B). In normal bathing solution (ACSF, $n = 16$ cells), hypoxia caused an initial suppression in the integral of spontaneous IPSPs by 10.7% (Fig. 8C, trace 2 and pre AD in Fig. 8D). After termination of the AD, the IPSP integral was significantly ($P<0.02$) augmented by 45% (Fig. 8C, trace, 3, and post AD in Fig. 8D). Spontaneous inhibitory activity gradually decreased during the observation period but was still significantly ($P<0.01$) enhanced by 16% of its control value after 15–30 min of reoxygenation. In order to exclude the involvement of any excitatory synaptic activity, NMDA and non-NMDA receptors were blocked by application of APV and CNQX, respectively ($n=5$ cells). Spontaneous IPSPs recorded in APV and CNQX did not differ in their appearance from IPSPs observed in normal ACSF, suggesting further an inhibitory synaptic character of these events. These experiments confirmed our observations in normal ACSF. The integral of spontaneous IPSPs was initially suppressed by 20.1%, followed by a phase of increased inhibitory synaptic activity (117.7%; filled bars in Fig. 8D). However, this pronounced increase was not significant at the $P<0.05$ level. Spontaneous IPSPs showed complete recovery within the observation period.

The characteristics of the AD recorded with potassium nitrate electrodes in normal ACSF (amplitude 52.7 ± 14.3 mV; onset latency 3.7 ± 1.1 min; duration at half-maximal amplitude 1.9 ± 0.7 min) were not significantly different from those of intracellular recordings with potassium acetate (see above). However, in comparison with the data obtained with potassium acetate electrodes in ACSF, addition of APV and CNQX to the bathing solution induced a significant ($P<0.02$) decrease in the AD amplitude (42.6 ± 9.5 mV). Latency (3.5 ± 0.7 min) and duration at half-maximal amplitude (1.93 ± 1.0 min) of the AD were unaffected by blockade of NMDA and non-NMDA receptors.

Developmental alterations in responsiveness to hypoxia

Age-dependent changes in the susceptibility of the neocortex to hypoxia were studied with FP responses to a double-stimulus protocol. In neocortical slices obtained from adult animals (>P28), the characteristic response consisted of a negative 1–4 mV d.c. deflection to the first stimulus and a 60–70% reduction of the response amplitude to the second stimulus due to concurrent activation of intracortical inhibitory pathways (Fig. 9A, period 1). This type of response was very stable over the observation period of 10 min (open symbols in Fig. 9B,C;

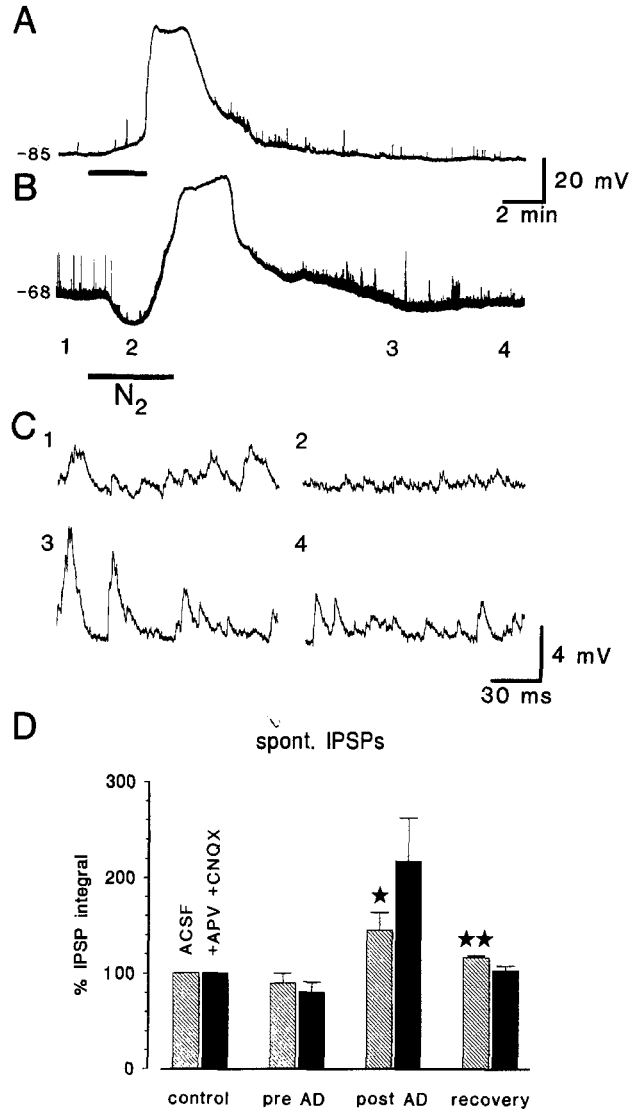

Fig. 8A–D. Hypoxia-induced changes of spontaneous IPSPs. **A, B** Response of two supragranular, regular spiking cells, recorded with 2 M potassium nitrate electrodes in normal bathing solution. Resting membrane potentials, $V_m=-85$ mV (**A**) and $V_m=-68$ mV (**B**). **B** Depolarizing spontaneous IPSPs are initially suppressed during the anoxic hyperpolarization (pre-AD; 2). In the early recovery period following the anoxic depolarization (post-AD), an increase in synaptic noise reflects an elevation of spontaneous IPSPs (3). **C** Characteristic sequence of spontaneous IPSPs recorded in the cell shown in **B** at times as indicated by *numbers* corresponding to those in **B** (1, control; 2, pre-AD; 3, post-AD; 4, recovery). **D** Percentage changes in the integral of spontaneous IPSPs recorded in artificial cerebrospinal fluid (*ACSF*; *shaded bars*, $n=16$) and in a bathing solution containing DL-amino-phosphonovaleric acid (*APV*) and 6-cyano-7-nitroquinoxaline-2,3-dione (*CNQX*; *filled bars*, $n=5$). *Bars on each column* indicate SEM. *Asterisks* symbolize significant differences: * $P<0.02$ ** $P<0.01$ level (Student's *t*-test)

$n=6$). During hypoxia, excitatory synaptic transmission declined by 41.7% and paired-pulse inhibition was completely blocked (Fig. 9A, period 2, filled symbols in Fig. 9B, C; $n=17$). Upon reoxygenation, excitatory and inhibitory synaptic transmission recovered to control values (Fig. 9A, period 3, B, C) and the latency of the half-time recovery was 5.5 min and 5.8 min, respectively.

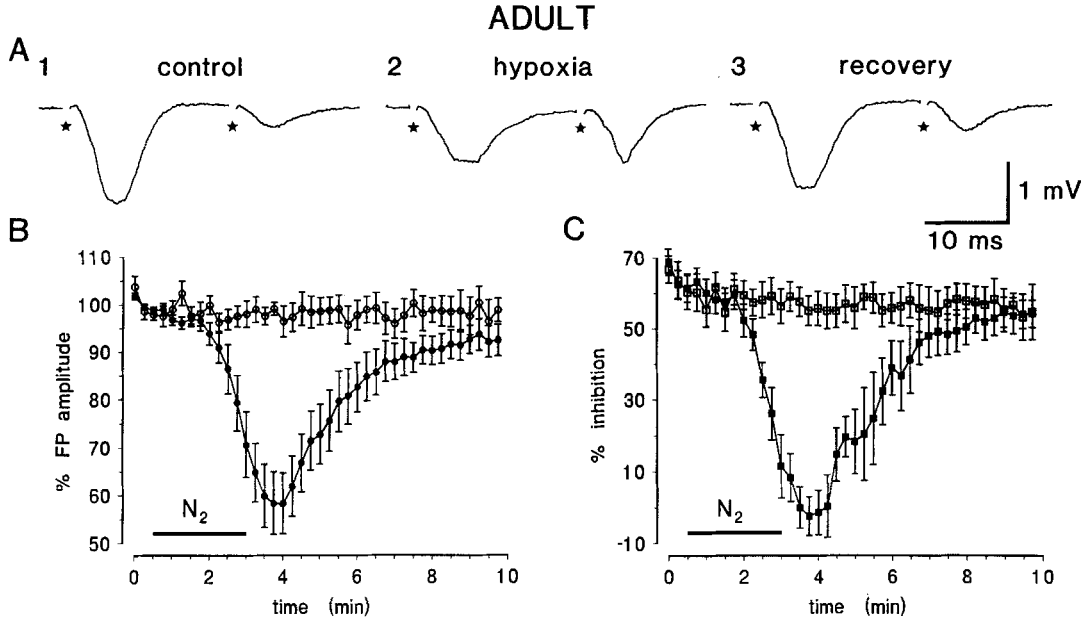

Fig. 9A-C. Hypoxia-induced modifications of field potential (*FP*) responses to paired-pulse stimulation in adult (>P28) neocortical slice (**A**). Hypoxia causes prominent reduction in excitatory synaptic transmission (**B**) and complete loss of paired-pulse inhibition (**C**). **A** Typical FP responses to double stimulus (*asterisks*) under control conditions (1), at the end of 2.5 min hypoxia (2), and during recovery phase (3). Note lack of paired-pulse inhibition in 2. **B** Mean FP responses to first stimulus under normoxic (*open circles*, $n=6$) and hypoxic conditions (*filled circles*, $n=17$). Hypoxia was induced by nitrogen aeration between 0.5 and 3.0 min (*horizontal bar* in **B** and **C**). **C** Efficacy of paired-pulse inhibition under normoxia (*open squares*, $n=6$) and hypoxia (*filled squares*, $n=17$). Note hypoxia-induced loss of inhibitory function and full recovery within observation period of 10 min. Some of the data shown in **B** and **C** have been published in a previous report (Luhmann and Heinemann 1992b). *Bars on each symbol* represent SEM

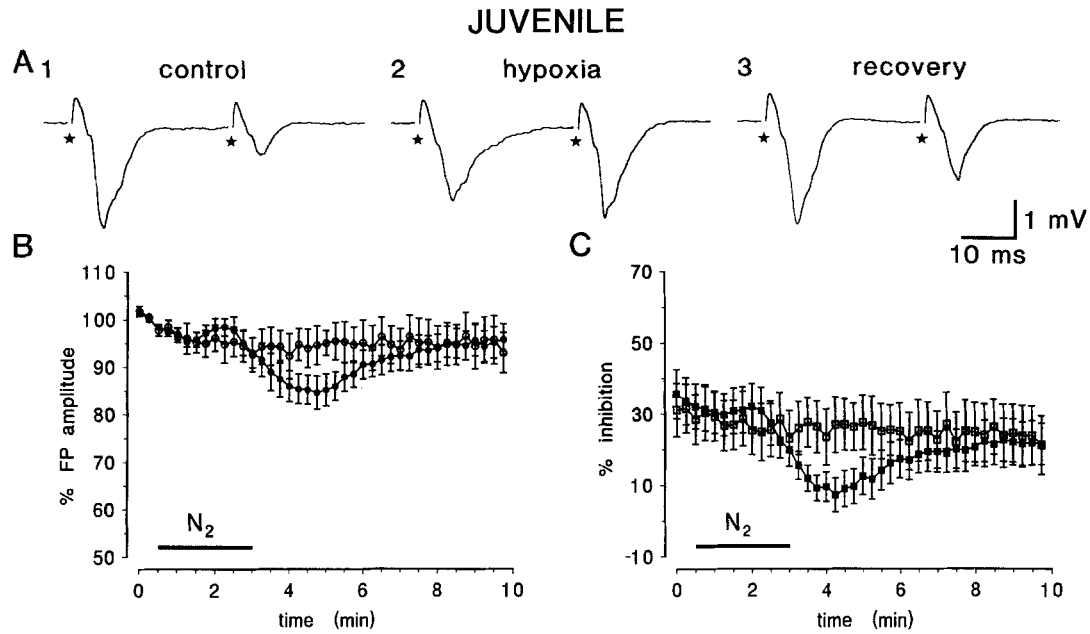

Fig. 10A–C. Effect of 2.5 min hypoxia on excitatory and inhibitory synaptic transmission in juvenile (P14-18) rat neocortex. **A** Characteristic response to double stimulus recorded in a P16 neocortical slice under control conditions (1), during hypoxia (2), and during recovery (3). **B** FP responses to first stimulus under normoxic (*open circles*, $n=4$) and hypoxic conditions (*filled circles*, $n=12$). **C** Paired-pulse inhibition in control recordings (*open squares*, $n=4$) and under hypoxia (*filled squares*, $n=12$). For further explanation see Fig. 9

In juvenile cortex (P14-18), paired-pulse inhibition was less intense than in adult animals (Fig. 10). Hypoxia caused a 15.4% reduction in excitatory synaptic transmission (Fig. 10A, period 2, B; $n=12$) and a decline in inhibition to 7.2% (Fig. 10A, period 2, C). Excitatory and inhibitory responses recovered within the observation period (Fig. 10A, period 3) and half-time recovery was 7.0 min and 8.0 min, respectively. A pronounced resistance to hypoxia could be detected for stimulus-evoked FP responses in young neocortical slices (P5-8). The FP

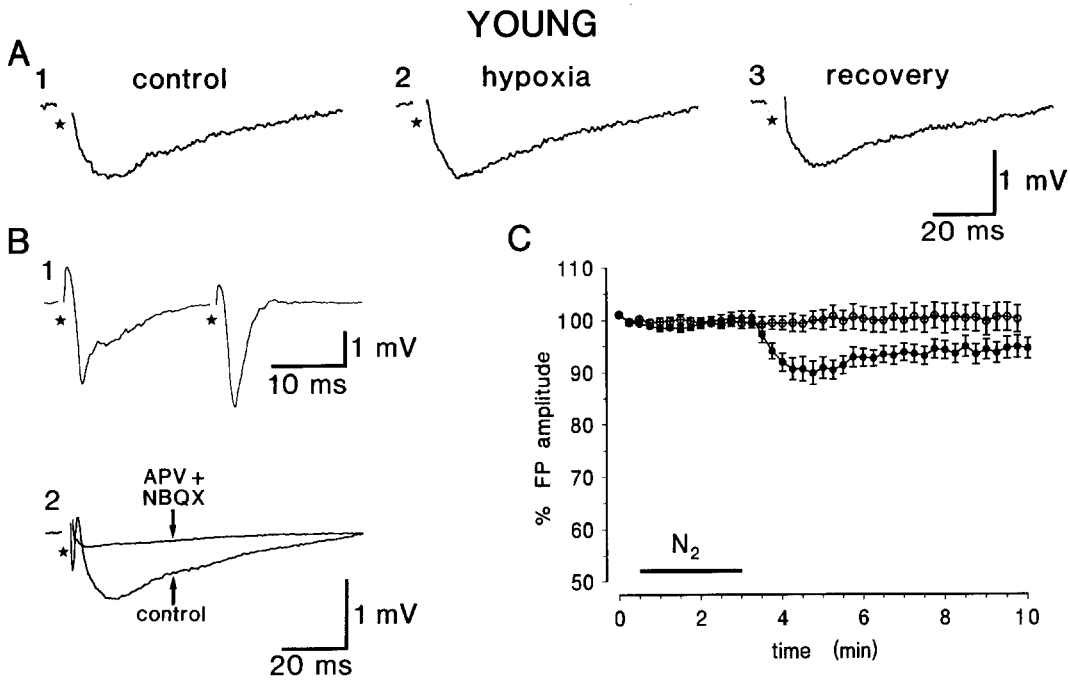

Fig. 11A–C. Hypoxia induces only minor changes in stimulus-evoked field potential responses in young (P5-8) neocortex. **A** Recordings in a P8 neocortical slice under control (1) and hypoxic conditions (2), and during recovery (3). **B** Lack of paired-pulse inhibition (1) and effect of local application of 300 μM APV + 100 μM NBQX on the evoked FP response (2) in a P8 neocortical slice. Traces in 2 are means of ten single responses. **C** FP responses under normoxic (*open circles*, $n=6$) and hypoxic conditions (*filled circles*, $n=10$). For details see Fig. 9

amplitude declined slightly during hypoxia, by 10.2% (Fig. 11A, period 2, filled circles in Fig. 11C; $n=10$), and slowly recovered within the observation period. Control measurements displayed a stable FP response under normoxic conditions (open circles in Fig. 11C; $n=6$). As expected from previous anatomical (Bähr and Wolff 1985; Miller 1986) and electrophysiological studies (Kriegstein et al. 1987; Luhmann and Prince 1991), inhibitory synaptic mechanisms were not present in this age group and notable paired-pulse suppression was absent (Fig. 11B, trace 1). In order to evaluate the involvement of antidromic activity in mediating the FP responses and the marked resistance of young cortex to hypoxia, excitatory synaptic transmission was blocked by local application of 300 μM APV and 50–100 μM NBQX ($n=8$ FPs) or 300 μM APV and 100 μM CNQX ($n=6$ FPs). After blockade of excitatory synaptic transmission, the mean FP response in young cortex declined by 91.7% from 1.56 ± 0.88 to 0.18 ± 0.35 mV ($n=14$; Fig. 11B, trace 2). The partial block of the FP responses by APV and CNQX or NBQX suggests an incomplete washing of these antagonists or an involvement of another excitatory amino acid receptor in this age group. However, most of the response seems to be mediated via the orthodromic pathway involving activation of the NMDA and AMPA/kainate receptor.

A comparison of the age-dependent increase in the susceptibility to hypoxia between young, juvenile, and adult neocortical slices is shown in Fig. 12, in which the amount of hypoxia-induced depression of the FP response is plotted against the latency of maximal suppression of the FP response. The magnitude of reduction of

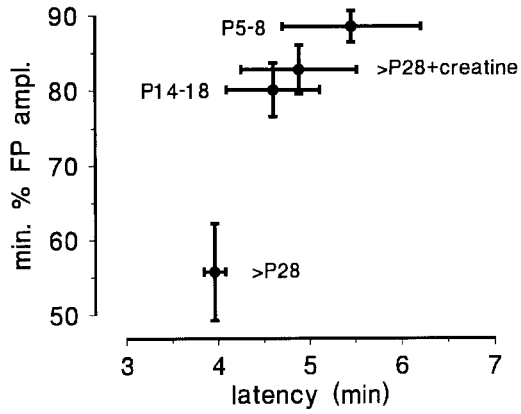

Fig. 12. The latency (*x*-axis) and the magnitude (*y*-axis) of hypoxia-induced maximal suppression of field potential (*FP*) responses. Data were obtained from measurements in young (*P5-8*, $n=10$), juvenile (*P14-18*, $n=12$) and adult (*>P28*, $n=17$) rat neocortical slices, as shown in Figs. 9–11. For comparison, results from a previous study (Luhmann and Heinemann 1992b) of creatine preincubated adult slices (*>P28 + creatine*, $n=12$) are included. *Horizontal and vertical bars on each symbol* indicate SEM of the response latency and amplitude, respectively

the FP response in adult cortex ($44.3 \pm 26.7\%$, $n=17$) was significantly larger than that in the juvenile ($19.9 \pm 12.3\%$, $n=12$; $P<0.01$) and young cortex ($11.5 \pm 6.6\%$, $n=10$; $P<0.001$). In addition, the latency of maximal suppression in the FP response is smaller in adult cortex (3.9 ± 0.5 min, $n=12$) than the latency in juvenile (4.6 ± 1.8 min, $n=12$) and young cortex (5.5 ± 2.4 min, $n=10$; $P<0.05$). In a previous report, we

demonstrated a pronounced protective effect in mature cortical slices by raising the intracellular concentration of high-energy phosphates after preincubating the slices in 25 mM creatine (Luhmann and Heinemann 1992b). Including these data in Fig. 12 illustrates, that the maintenance of sufficiently high intracellular ATP concentrations may represent an important factor in the age-dependent sensitivity to hypoxia. In creatine-treated adult slices, the amount of synaptic suppression ($17.2 \pm 11.2\%$, $n = 12$) was significantly ($P < 0.001$) reduced as compared to untreated adult controls. In addition, the latency of the maximal suppressed synaptic response ($P < 0.01$) was increased, suggesting a distinct protective effect of creatine incubation against anoxia (Fig. 12).

Discussion

The experiments described in this report were designed to investigate the mechanisms that lead to the hypoxia-induced reduction of GABAergic inhibition and age-dependent alterations in the responsiveness to hypoxia. The striking response to sufficiently long hypoxic periods in vivo (Speckmann et al. 1973) and in vitro (Balestrino et al. 1989; Rader and Lanthorn 1989; Silver and Erecinska 1990) is a prominent AD, similar to the spreading depression (SD) described by Leão (1944, 1947). However, in contrast to SD (Mody et al. 1987), the AD amplitude is not affected by competitive NMDA receptor antagonists (Kral et al. 1993), and the recovery phase following an AD is characterized by a transient increase in spontaneous synaptic activity, which is not necessarily detectable with extracellular recording techniques. These data indicate that the basic mechanisms underlying the SD and AD are functionally different. Our observation that APV and CNQX induced a significant reduction in the AD amplitude suggests that non-NMDA receptors (Koh et al. 1990; Kohmura et al. 1991; Sheardown et al. 1990) or the concurrent activation of NMDA and non-NMDA receptors (Clark and Rothman 1987; Frandsen et al. 1989; Michaels and Rothman 1990; Rothman 1983) play an important part in mediating ischemic depolarization.

In addition to the prominent AD, with a mean amplitude of 55 mV, hypoxia initially evokes a small gradual depolarization by 3–9 mV in a number of neocortical neurons (Luhmann and Heinemann 1992b; Rosen and Morris 1991). The majority of rat CA3 hippocampal neurons respond to transient hypoxia also with an initial depolarization with a mean of 8 mV (Ben-Ari 1990a,b). Blockade of synaptic transmission with tetrodotoxin (TTX) reduced (Luhmann and Heinemann 1992b; Rosen and Morris 1991) or blocked (Ben-Ari 1990a,b) the initial small depolarization. In addition, in the CA3 area this response is blocked by kynurenate, indicating that a hypoxia-induced increase in glutamate release mediates the postsynaptic depolarization in this region. An involvement of ATP-dependent K^+ channels in mediating this presynaptic mechanism has been described by Ben-Ari (1990a, 1990b). However, in the hippocampal CA1 region and in the neocortex, an initial depolarization is absent or of much smaller amplitude (Clark and Rothman 1987; Fujiwara et al. 1987; Hansen et al. 1982; Langmoen and Berg-Johnsen 1988; Leblond and Krnjević 1989; Luhmann and Heinemann 1992b; Reiner et al. 1990), indicating that hypoxia-induced glutamate release is less powerful. The prominent initial response to hypoxia in these brain regions is a hyperpolarization, which is mediated by an increase in a Ca^{2+}-activated (Leblond and Krnjević 1989) and/or ATP-dependent K^+ conductance (Grigg and Anderson 1989; Luhmann and Heinemann 1992b; Mourre et al. 1989; Tromba et al. 1992). Recent observations by Zhang and Krnjević (1993) in CA1 pyramidal neurons using the whole-cell patch clamp recording technique in 400 µm thick slices indicate that the intracellular ATP concentration plays a crucial role in mediating the anoxic hyperpolarization and the hypoxia-induced reduction in input resistance. However, further analyses are required to elucidate the underlying mechanisms.

Suppression of inhibition

The significant reduction of stimulus-evoked IPSPs and paired-pulse inhibition during and particularly after hypoxia indicates a pronounced impact of hypoxia on intracortical GABAergic mechanisms. Several observations demonstrate an enhanced sensitivity of the inhibitory system:

1. Paired-pulse inhibition was completely blocked at a time when excitatory synaptic transmission was reduced by only 41.7% (Fig. 9). A pure decrease in the excitatory drive is insufficient to explain the complete blockade of synaptic inhibition, since a reduction in the excitatory drive by 40% still evokes pronounced inhibition (Fig. 1B).

2. Previous observations have shown that the hypoxia-induced decrease in paired-pulse inhibition is not dependent on the stimulus intensity (Luhmann and Heinemann 1992b).

3. During brief hypoxia (4–6 min), the initial decrease in the IPSPs was faster than the EPSPs and recovery was slower for inhibitory than for excitatory synaptic transmission (Rosen and Morris 1993). In addition, following an AD, EPSPs recovered earlier than hyperpolarizing IPSPs (Fig. 5). The lack of hyperpolarizing IPSPs in the early post-AD period may be caused by the ionic imbalance due to increases in extracellular potassium and intracellular chloride and may lead to a late functional recovery of GABAergic inhibition. It has been shown that phosphorylation of the $GABA_A$ receptor is necessary to maintain $GABA_A$ receptor function and to prevent the "rundown" of $GABA_A$-mediated inhibition (Chen et al. 1990; Stelzer et al. 1988; for reviews, see Leidenheimer et al. 1991; Raymond et al. 1993). Hypoxia probably influences this phosphorylation process and may subsequently decrease the efficacy of inhibitory mechanisms during and after oxygen deprivation. In addition, hypoxia may alter the desensitization kinetics of the $GABA_A$ receptor.

4. The recovery of the f- and l-IPSP upon short-term hypoxia followed a different time course, indicating that

$GABA_A$ and $GABA_B$ receptors are not equally affected by oxygen deprivation (Luhmann and Heinemann 1992b).

5. Recent experiments using long-term post-AD recovery times and the noncompetitive NMDA antagonist dextromethorphan demonstrate a complete recovery of excitatory synaptic transmission whereas intracortical inhibitory mechanisms are irreversibly impaired (Luhmann and Scheid, in preparation).

Anatomical (Sloper et al. 1980), immunocytochemical (Esclapez and Trottier 1989; Houser et al. 1986; Sloviter 1987; Takahashi et al. 1991) and biochemical data (Löscher et al. 1981; Manyam et al. 1980) indicate a selective vulnerability of the GABAergic system to ischemia, possibly the primary cause of epilepsy (for review, see Ribak 1991; Roberts 1986), but this hypothesis is currently being questioned (for review, see Meyer 1989; Schmidt-Kastner and Freund 1991). In vitro studies have demonstrated with electrophysiological techniques that inhibitory synaptic transmission has a higher susceptibility to hypoxia than do excitatory processes (Fujiwara et al. 1987; Hershkowitz et al. 1993; Krnjević et al. 1991; Leblond and Krnjević 1989; Luhmann and Heinemann 1992b; Rosen and Morris 1993). The data presented in this report give some indications of the functional mechanisms underlying this sensitivity to hypoxia. Hypoxia causes a significant depolarizing shift in the reversal potentials of stimulus-evoked f- and l-IPSPs by 24.4 and 14.9 mV, respectively. In the post-AD period, when EPSPs are partially recovered, hyperpolarizing IPSPs are still absent (Fig. 5E). The depolarizing shift in E_{IPSP} is probably caused by the massive disturbance in ion homeostasis during hypoxia (for review, see Hansen 1985). The deficiency in brain energy metabolism leads to interference of neuronal ion transport mechanisms with a subsequent rise of intracellular calcium, chloride, and sodium, and extracellular potassium. Under the condition of elevated intracellular chloride, $GABA_A$ receptor activation may not necessarily have a neuroprotective effect (Ylinen et al. 1991) by hyperpolarizing the postsynaptic membrane, but may rather depolarize the cell (Yuste and Katz 1991; Segal 1993).

Presynaptic mechanisms, such as a decrease in GABA release, may contribute to a functional impairment of the intracortical inhibitory system (Rosen and Morris 1993). However, a significant reduction in K^+-stimulated GABA release could not be detected in the CA1 hippocampal region of rats subjected to global cerebral ischemia (Johansen et al. 1991). In the present study spontaneous IPSPs showed a different response pattern to hypoxia as compared to stimulus-evoked IPSPs. Due to the high permeability ratio of nitrate (2.1 as compared to 1.0 for chloride; Bormann et al. 1987) and the likely difference in the efficiency of active extruding mechanisms for chloride (Lux 1971; Misgeld et al. 1986; Thompson et al. 1988, 1989) and nitrate, depolarizing spontaneous IPSPs could be reliably observed with electrodes containing potassium nitrate. After an initial suppression during the early hypoxic period, spontaneous IPSPs were significantly augmented in the post-AD phase. This long-term enhancement of spontaneous IPSPs observed in control bathing solution (Fig. 8D) probably does not reflect a steady diffusion of nitrate into the cell because data were obtained after the spontaneous IPSPs reached an apparent steady state. In addition, a long-term increase in IPSP integral could not be detected in our recordings under normoxic conditions and in ACSF containing APV and CNQX. Therefore we consider a long-term increase in spontaneous IPSPs due to continuous diffusion of nitrate into the cell as unlikely. The exact mechanisms underlying this augmentation (increase in IPSP amplitude and/or frequency) are hard to investigate in detail under current-clamp conditions, especially with prominent changes in the resting membrane potential during the post-hypoxic phase (see slow membrane deflection following the AD in Figs. 4B, trace 2, 8A,B). We consider it rather unlikely that the post-AD increase in spontaneous inhibitory activity is caused by a rise in the frequency of action potential dependent IPSPs. Under normal in vitro conditions, cortical neurons do not reveal any spontaneous action potentials and spontaneous IPSCs have been shown to be TTX-resistant (Otis et al. 1991; Otis and Mody 1992), indicating that they are not driven by synaptic activity. In addition, none of the interneurons studied in this report showed any spontaneous action potentials during or after the hypoxia. On the basis of these observations, we favor the hypothesis that hypoxia induces an increase in action potential independent spontaneous IPSPs. Hypoxia causes a rise in the presynaptic Ca^{2+} influx (Young and Somjen 1992) and Ca^{2+} extrusion mechanisms, such as the Ca^{2+} pump and the Na^+-Ca^{2+} exchanger (Stys et al. 1992) may be functionally impaired (for review, see Waxman et al. 1991). The elevation in the presynaptic Ca^{2+} concentration leads to increased exocytosis and synaptic noise. This rise in spontaneous activity may also lead to depletion of presynaptic transmitter stores and possibly a subsequent reduction of stimulus-evoked IPSPs.

Our observations on morphologically or functionally characterized interneurons do not support the hypothesis of a selective susceptibility of GABAergic cells. Although we did not verify the GABAergic character of these cells by immunohistochemical staining techniques, the electrophysiological (short action potentials, no or weak spike frequency adaptation) and morphological parameters (small somata, nonspiny dendrites) suggest that these neurons are local inhibitory interneurons (McCormick et al. 1985). This structural-functional classification is in agreement with a recent report by Kawaguchi (1993) on rat frontal cortex, demonstrating that non-spiny interneurons reveal the characteristic physiology of fast spiking cells. However, in another population of nonpyramidal cells, broader action potentials (>0.8 ms), prolonged afterhyperpolarizations, and apparent spike frequency adaptation could be observed (Kawaguchi 1993).

Our observations on the effects of transient hypoxia on interneurons and spontaneous IPSPs neither support the hypothesis of a selective susceptibility of GABAergic cells nor a selective functional impairment of presynaptic GABA release mechanisms, but rather require hypoxia-sensitive alterations in pre- and postsynaptic processes.

The prominent reduction of stimulus-evoked IPSPs, at a time when spontaneous IPSPs are augmented, suggests that postsynaptic mechanisms play an important role in mediating the enhanced susceptibility of the GABAergic system to hypoxia. Ischemia-induced changes in the $GABA_A$ receptor-chloride-channel complex have been recently reported in the Mongolian gerbil by Mileson et al. (1992). In the photothrombosis model of focal cerebral ischemia (Watson et al. 1985), a reduction of intracortical inhibitory mechanisms has been observed under in vivo conditions (Domann et al. 1993) and this ischemia-induced suppression of inhibitory function has been attributed to a reduction of postsynaptic $GABA_A$ receptors (Zilles and Witte, personal communication). Further evidence of a postsynaptic mechanism comes from the work on kindling epileptogenesis in rat hippocampus. Anatomical (Kamphuis et al. 1989) and physiological (Kamphuis et al. 1991a) data indicate that the kindling-induced hyperexcitability is caused by a reduction in the efficacy of the GABAergic system. This disinhibition is rather mediated by modifications of the postsynaptic GABA receptor complex than by a presynaptic mechanism because GABA release is significantly enhanced in kindled hippocampus (Kamphuis et al. 1990, 1991b). Recent molecular biology results from the same laboratory indicate that kindling induces a significant reduction in the expression of the $GABA_A$-$\beta 2$ subunit (Lopes da Silva, personal communication). Such modifications in the densitiy and/or function of postsynaptic $GABA_A$ receptors with a subsequent decrease in GABAergic inhibition would not have been recognized with conventional neuroanatomical techniques, because inhibitory interneurons and GABAergic synaptic terminals would be possibly unchanged. A modification in the expression pattern of the $GABA_A$ receptor or one of its subunits (Wisden et al. 1992), as it has been observed in schizophrenic patients (Benes et al. 1992) and after long-term exposure to diazepam (Heninger et al. 1990), may also operate as a possible mechanism to induce a long-term decrease in the sensitivity of postsynaptic GABA receptors after cerebral hypoxia. Finally, dephosphorylation of the GABA receptor during hypoxia may alter its functional properties (for review, see Raymond et al. 1993). Further studies are required to clarify the role of postsynaptic GABAergic mechanisms in ischemia-induced functional and structural deficits.

Resistance of immature cortex to hypoxia

The properties of the FP responses reported in this study are in good agreement with previous observations in adult rat neocortex by Aroniadou and Teyler (1991). Our measurements on stimulus-to-peak FP responses in immature and adult cortex (5.2–6.5 ms) are in accordance with their results obtained in mature rat visual cortex (5–8 ms). In agreement with previous reports (Cherubini et al. 1989; Hansen 1977), we observed a significant age-dependent increase in the sensitivity of rat neocortex to hypoxia (for review, see Hansen 1985). Both the magnitude and the latency of hypoxia-induced synaptic transmission were clearly dependent on the age of the animal. A pronounced resistance to oxygen deprivation could be detected in young and juvenile cortex. Recent experiments in this laboratory on the responsiveness of immature cortex to long periods of hypoxia (up to 1 h) also suggest that young and juvenile slices tolerate much longer periods of oxygen deprivation than adult tissue. However, once an AD is triggered, the magnitude of the extracellular d.c. deflection and the extracellular calcium decrease is significantly larger in immature than in adult cortex (Luhmann 1993).

Several factors may contribute to the resistance of immature brain to ischemia:

1. Young neurons may have smaller demands for energy-consuming processes (Duffy et al. 1975; Kawai et al. 1989), such as intracellular transport mechanisms and ion pump activity to preserve intracellular ion homeostasis. The smaller somatic size, axonal arborization and dendritic branching pattern of immature neurons would support this hypothesis.

2. Under hypoxic or hypoglycemic conditions, the immature brain is capable to maintain sufficiently high concentrations of intracellular energy resources via anaerobic glycolysis (Hansen and Nordstrom 1979; for review, see Hansen 1985; Himwich 1951).

3. Postnatal alterations in the functional state of the GABA receptor or its subunits may profoundly alter the sensitivity to hypoxic-ischemic influences (for heterogeneity of $GABA_A$ receptor subunits see Vicini 1991; Wisden et al. 1992).

4. Immature neurons are capable of down-regulating within minutes the density of sodium channels after prolonged channel activation, which enables them to adapt rapidly to a massive hypoxia-induced sodium influx and to prevent irreversible functional deficits (Dargent and Couraud 1990).

Recent observations by Erdö et al. (1991) indicate that under ischemic conditions GABA may act as a cofactor in mediating excitotoxic cell death. This hypothesis would demand a decreased sensitivity to hypoxia in brain tissue that shows a lower concentration of GABA. Anatomical (Bähr and Wolff 1985; Miller 1986) and electrophysiological data (Kriegstein et al. 1987; Luhmann and Prince 1991) indicate that the GABAergic system is still immature in the young and juvenile age group studied in this report.

Although the mechanisms described above may contribute to the insensitivity of immature cortex to hypoxia, our observations in creatine preincubated adult slices suggest that the hypoxia-induced depletion of intracellular high-energy phosphates is predominantly important (Luhmann and Heinemann 1992b). In rat hippocampal slices, creatine incubation caused a fourfold rise in phosphocreatine content (Lipton and Whittingham 1982; Whittingham and Lipton 1981). As in hippocampus, this pretreatment had a prominent protective effect on the preservation of excitatory and inhibitory synaptic transmission during hypoxia in adult neocortical slices. These data suggest that the trigger for the induction of hypoxia-induced functional deficits is a depletion of intracellular

energy reserves. For developmental reasons described above, the immature cortex is probably far more efficient in maintaining these energy sources.

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