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Anoxic Block of GABAergic IPSPs*

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In rat hippocampal slices GABAergic IPSPs are very rapidly suppressed by anoxia (in < 2 min). Both early ($GABA_A$) and late ($GABA_B$) components are affected. After reoxygenation, the IPSPs recover, but only slowly and not always completely. Iontophoretic applications of GABA or baclofen indicated no major depression of responses during anoxia. It is therefore unlikely that the anoxic suppression of IPSPs is caused by desensitizations of GABA receptors. A more probable explanation is a failure of GABAergic neurons to release GABA from inhibitory nerve terminals.

KEY WORDS: Anoxia; inhibitory synaptic potentials (IPSP); GABA; baclofen; hippocampus.

INTRODUCTION

The full significance of the discovery of GABA in the brain (1) became evident when it turned out that GABA has all the properties of the natural inhibitory transmitter in the cortex (2,3), and that vast numbers of central neurons are rich in glutamic-acid decarboxylase, and therefore probably GABAergic inhibitory neurons (4). GABAergic transmission is now recognized as the most widely distributed mechanism of synaptic inhibition, throughout the CNS.

An interesting property of GABAergic IPSPs in hippocampal slices—first observed by Fujiwara et al. (5), and subsequently confirmed by Leblond and Krnjević (6)—is their marked sensitivity to anoxia. One of the earliest effects of anoxia is the suppression of IPSPs, at a time (30–60s) when EPSPs show no comparable depression. Clearly, such a selective loss of IPSPs cannot be explained by a general interference with synaptic transmission.

A selective depression of GABAergic transmission could be produced by either a presynaptic or a post-synaptic mechanism: the former a block of GABA release from inhibitory terminals; the latter a loss of post-synaptic sensitivity to GABA.

A post-synaptic change in GABA-sensitivity would be in keeping with, on the one hand, evidence that GABA-receptors are desensitized by intracellular Ca^{2+} (7–9) and on the other, a probable increase in intraneuronal free Ca^{2+} during anoxia (6, 10–12). Another possible link between anoxia and a run-down of GABA sensitivity is depletion of ATP, because $GABA_A$ receptors require phosphorylation for their functional integrity (9).

In the present experiments, we have further examined the anoxic suppression of IPSPs in hippocampal slices and tested for a possible loss of post-synaptic sensitivity to GABA.

EXPERIMENTAL PROCEDURE

Conventional hippocampal slices were prepared from the brains of Sprague-Dawley rats. They were superfused with artificial cerebrospinal fluid (ACSF) in an interface chamber, at 33–34°, as described

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in detail previously (6). Anoxia was produced by changing the aerating gas from 95% O_2 to 95% N_2, keeping CO_2 constant at 5%. Intracellular recordings from CA1 pyramidal layer cells were made by current clamp or by single-electrode voltage clamp, with 3 M KCl, K acetate or K methylsulphate-filled microelectrodes. GABA or baclofen (β(4-chlorophenyl)-GABA) was applied by "push-pull" microiontophoresis from 3-barrelled micropipettes filled with concentrated GABA (1 M, pH 4.5 cf. ref. 2), and/or (±) baclofen (10 mM cf. ref. 13), as well as 1 M NaCl (for the return current). Leakage of GABA or baclofen was usually prevented by a "backing current" of 10–25 nA. Synaptic responses were evoked by stimulating electrodes placed in the stratum radiatum. Input conductance (G_N) was measured by applying constant hyperpolarizing current pulses (during current-clamp) or hyperpolarizing commands (during voltage-clamp).

RESULTS

In the first set of experiments, we confirmed that anoxia very quickly and consistently abolishes IPSPs.

Anoxic block of IPSPs

Time Course. The rapid block of IPSPs is illustrated in Fig. 1A. Initially, this CA1 neuron (V_m −62 mV), recorded with a K-acetate electrode, was firing "spontaneously" and a large and prolonged hyperpolarizing IPSP was evoked at 5s intervals—the IPSP is seen first on fast paper trace (at left) and then as regular downward

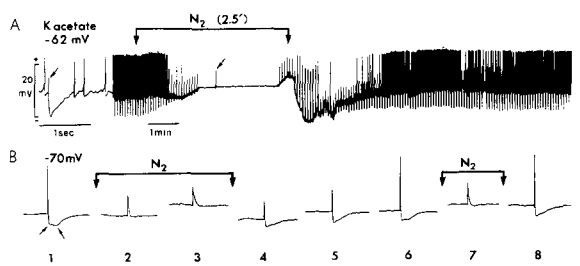

Fig. 1. ISPSs of CA1 neurons are rapidly blocked by anoxia. *A.* continuous paper trace of spontaneous firing and large hyperpolarizing IPSPs evoked by regular, constant intensity stratum radiatum stimulation (at small arrow in accelerated trace at left). Time during which O_2 was replaced by N_2 is indicated by bold arrows: note progressive reduction in IPSP amplitude (downward deflections on slower trace), as well as slow hyperpolarizing shift and cessation of firing. Accelerated trace *during* anoxia shows absence of IPSP following stimulation, at second small arrow. Intracellular recording was with K acetate electrode. *B.* Individual IPSPs recorded from another CA1 neuron with K acetate electrode. In this case, control trace at left (1) shows clear distinction between early and late IPSP (arrows). Traces 2 and 3 were recorded 100 s and 200 s respectively after start of 4 min period of anoxia: note again marked suppression of IPSP (both early and late phases), in spite of depolarizing shift, and increased duration of EPSP. Post-anoxic recovery is illustrated by traces 4 (2.5 min), 5 (4.5 min) and 6 (10 min post anoxia). Note slow recovery, especially of late phase. A second 3 min anoxic test is illustrated by trace 7 (at 2.5 min) and the subsequent partial recovery in 8 (at 6 min).

deflections on slower trace. Anoxia (between arrows) had its typical actions (5,6): first an early and transient small depolarization, at 30s turning into the characteristic hyperpolarization, associated with disappearance of spontaneous firing. The IPSPs began to diminish rapidly after 30s of anoxia and vanished by 90s. A second, slow phase of depolarization then became evident, during which acceleration of the trace showed no sign of any IPSP (though V_m was 1–2 mV more depolarized than initially). Reoxygenation was followed by the common phase of post-anoxic hyperpolarization (5,6), and then a slow return to the initial state. Although IPSPs began to reappear 1 min post-anoxia, their recovery was slow, being only 90% complete after 8 min. A slow, possibly incomplete recovery of IPSPs (even after 10–12 min) was a consistent finding (see also Figs. 4D in (5) and 3C in (6)), which was in contrast to the rapid, full and well-maintained recovery of EPSPs.

Early and Late Components of IPSP. It is now well-known that GABA activates at least two kinds of receptors, of A- and B-type (14,15), the latter being probably responsible for the slower, and very prolonged phase of hippocampal IPSPs (16,17). In the previous experiments on anoxia (5,6), no attempt was made to distinguish between these two components of IPSPs. Because the early Cl-mediated phase cannot be readily identified when recording with KCl electrodes, K-acetate or K-methylsulphate electrodes were used in several experiments. Under these conditions, the early and late components of IPSPs were usually clearly evident (as in Figure 1B).

In all cases, anoxia produced a similar, near-complete suppression of both early and late IPSPs—as in Figure 1B. Note in this Figure the persistence of EPSPs during anoxia, whose duration was prolonged (1B3), presumably owing to disappearance of the overlapping early IPSP; and also the apparently slower recovery of the late IPSP (1B5 and 6).

Anoxia Suppresses Inhibitory Conductance (G) Increase. In order to confirm that anoxia abolishes the primary mechanisms of the IPSP, the increase in G_d, mediated by $GABA_A$ receptors and the increase in G_K, mediated by $GABA_B$ receptors—and does not simply mask the IPSP by changing either V_m or the IPSP reversal potential—the following tests were made. Voltage-dependent reductions of IPSPs during anoxia were controlled by bringing V_m back to its initial pre-anoxic level. For example, Figure 2A shows a typical anoxic effect; first a transient depolarization and then marked hyperpolarization, during which the large negative IPSP nearly disappeared and the EPSP was much increased (as would be expected). When sufficient depolarizing

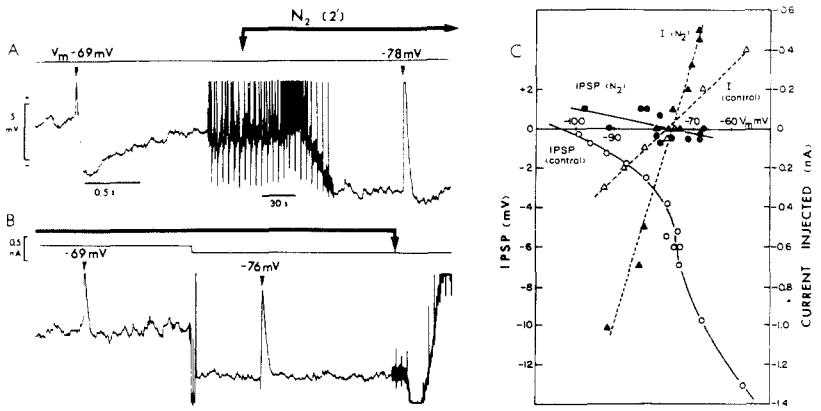

Fig. 2. Suppression of IPSPs is not secondary to voltage and conductance changes produced by anoxia. Recording with KCl microelectrode from CA1 neuron shows in *A*, prolonged hyperpolarizing IPSPs evoked by stratum radiatum stimulation (first on accelerated trace, and then on much slower trace). During 2 min of anoxia there was a transient depolarization and increase in firing, and then a marked (−9 mV) hyperpolarization, associated with a disappearance of IPSPs and spontaneous firing, Note large enhancement of EPSP in faster trace at right. *B* is continuous with A: at left in *B*, a small injection of depolarizing current (monitored on upper trace) returned potential (V_m) to initial level and greatly reduced amplitude (but not duration) of EPSP, but IPSP remained absent. Depolarizing current was then switched off, shortly before end of anoxia, which was followed by usual post-anoxic hyperpolarization and then a depolarizing overshoot (again on slower traces). *C* Graph of data from same cell showing IPSP as function of V_m (circles: open are control IPSPs; closed, IPSPs during anoxia (N_2) - latter points are fitted by linear regression), as well as current/voltage data (steady state values) obtained at same time (triangles: open are control values, closed, values during anoxia).

current was injected to return V_m to −69 mV (Figure 2B, traces continuing from 2A), the IPSP did not return, though the EPSP became smaller.

Similar observations were made in 20 cells. In addition, systematic measurements of IPSPs were done for different values of V_m. Plots of such data give an indication of the relative increase in conductance during the IPSP ($\Delta G/G_N$). As can be seen in Fig. 2C (same cell as in 2A,B) there is a steep but non-linear relation between Vm and IPSP amplitude (measured at 100 ms), with a reversal potential near −100 mV. The slope is relatively flat for V_m < −80 mV—largely because of inward rectification—and very steep near −70 mV (600 μV/mV). Thus, the inhibitory conductance change—and therefore inhibition—would be most effective in the region of the resting potential. In contrast, the black circles shows only minimal IPSPs during anoxia, with an only just significant voltage-dependence (40 ± 15 μV/mV). Such a large reduction in slope (to ~1/15 of control) cannot be accounted for by the simultaneous 3–5-fold increase in steady state G_N during anoxia (cf. open and closed triangles in Figure 2C).

GABA Responses During Anoxia. All the tests of GABA were done on cells recorded with KCl electrodes, and therefore giving at least partly depolarizing responses to GABA. The effects of anoxia were examined only when stable submaximal responses were produced by constant iontophoretic doses of GABA, applied at regular intervals. Thus, in Figure 3, control tests of GABA caused a depolarization by ~10 mV and a 100% increase in conductance (A). During anoxia (B), there was a small depolarization (by 2 mV) and marked rise in G_N (also by 100%); although GABA produced a smaller depolarization (by 7 mV), the increase in G_N was 3-fold greater than before.

Four similar tests during *current-clamp* recording (from 3 cells) showed consistent increases in GABA-evoked conductance change (by 59 to 460%) with a mean increase of +172%). Because voltage-dependent changes in G_N complicate the interpretation of these findings, four further tests were performed during *voltage-clamp* on two neurons, in the presence of tetrodotoxin.

Figure 4 illustrates such an experiment. The membrane potential was held at −70 mV, and −20 mV pulses were applied at regular intervals to monitor changes in G_N. A 2 min period of anoxia (A) generated a clear, but only briefly sustained outward (upward) current shift, and a progressive increase in G_N. Upon reoxygenation, there was another temporary outward shift (corresponding to post-anoxic hyperpolarization (5,6)) while G_N quickly returned towards the initial level. The lower traces (in B) illustrate the inward currents and increase in G_N evoked by GABA, before (at left), during a period of anoxia (in middle), and after recovery (at right). It is clear that the effects produced by GABA were not suppressed but rather were *enhanced* by anoxia. In four similar tests, anoxia produced only relatively small changes in GABA-evoked conductance, ranging from +11% to −14%, with a mean of −3.0%.

These results failed to provide any evidence that

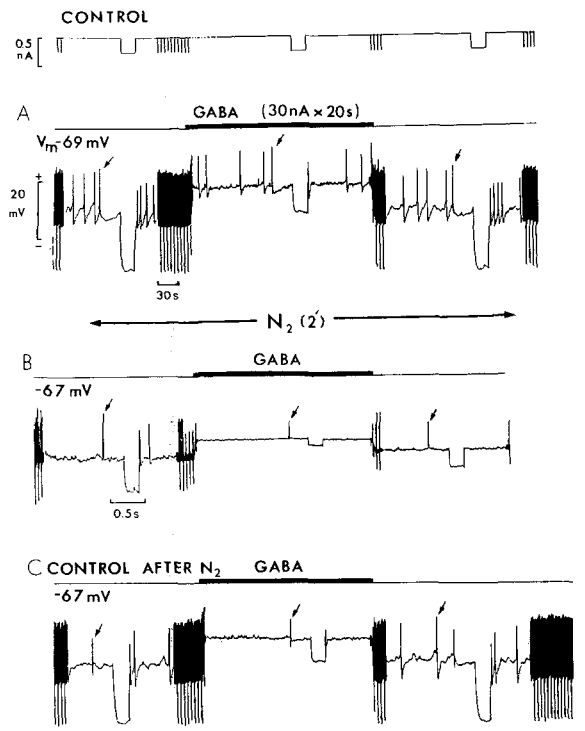

Fig. 3. Responses evoked by GABA applications are *not* suppressed by anoxia. Intracellular recording from CA1 neurons was with KCl electrode, and GABA was released by push-pull iontophoresis from an extracellular 3-barrelled micropipette: intensity and duration of iontophoretic currents are indicated. Input resistance was monitored by injecting 200 ms pulses of hyperpolarizing current, recorded in uppermost trace (for *A*). Paper traces were temporarily accelerated before, during and after GABA application to display potentials - including IPSPs evoked by stratum radiatum stimulation (arrow) - in greater detail. Initial resting potential is indicated. *A.* During GABA release, note marked depolarization and fall in input resistance, as well as smaller IPSP. *B.* After 90s of anoxia, there was a small depolarization and large reduction in resistance, and IPSPs are invisible; but GABA still causes a sharp depolarization and an even greater resistance drop. *C.* In subsequent control run, there is still little sign of the IPSP.

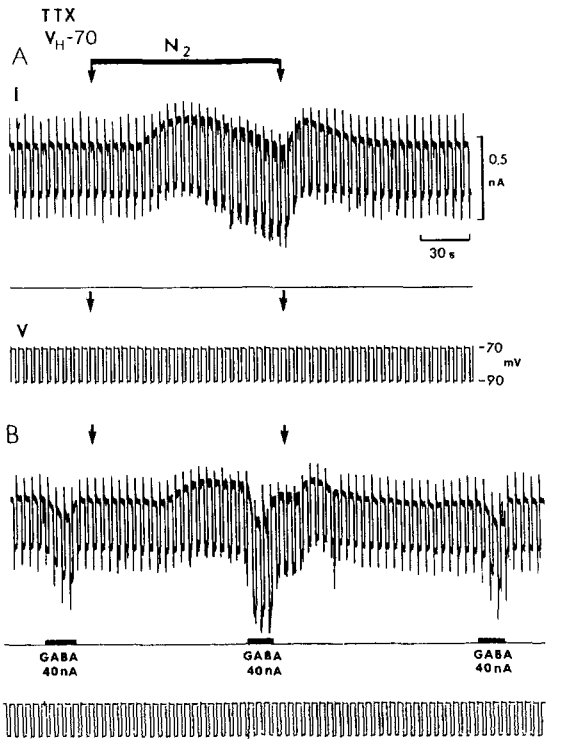

Fig. 4. Anoxia also fails to suppress GABA-evoked inward current and conductance increase (single-electrode (KCl) voltage-clamped recording from CA1 neuron in presence of 1 μM tetrodotoxin). Hyperpolarizing (−20 mV) commands, lasting 2s, were applied at 5s intervals throughout to monitor changes in conductance. *A*, upper trace shows transient outward shift in membrane current and gradual conductance increase during 2 min of anoxia (lower trace is clamp voltage). *B.* GABA applications (as indicated below) caused sharp inward shifts and increase in conductance before, during, and after anoxia. Note somewhat enhanced effects of GABA during anoxia.

GABA becomes substantially *less* effective during anoxia.

Baclofen Responses During Anoxia. In an attempt to detect possible selective changes in $GABA_B$-receptor sensitivity, (±) baclofen was applied instead of GABA in four experiments. The effects of baclofen, however, were slower, less pronounced and less consistent than those of GABA, especially with regard to changes in G_N. Because of the relatively large increase in V_m and G_N evoked by anoxia, only in two cells was it possible to quantify the effects of baclofen during anoxia. In these current-clamp recordings, anoxia appeared to *enhance* baclofen-evoked changes in V_m and G_N (by 33 and 180% respectively).

DISCUSSION

In these experiments, we have confirmed that IPSPs in CA1 neurons are highly susceptible to anoxia, a virtual disappearance of IPSPs being one of the earliest manifestations of the anoxic state. Anoxia suppresses both the early and the late components of IPSPs, that are mediated respectively by $GABA_A$-receptors and Cl^- channels, and $GABA_B$-receptors and G-protein-coupled K^+-channels (14).

The fact that two such very different inhibitory mechanisms are similarly affected by anoxia is more in keeping with a block of GABA release than a block of GABA action. In agreement with this idea, all the tests of GABA itself, applied directly by microiontophoresis, showed no evidence of a significant loss of efficacy during anoxia.

Indeed, the current clamp recordings indicated a

potentiation of GABA action. Could this perhaps be due to the fall in pH (18) observed in slices during anoxia (19), or a facilitatory action of glutamate (20), also released during anoxia (21)? Another possibility might be some form of synergism between GABA-activation of G_{Cl} and G_K and Ca-activation of G_{Cl} and G_K (both a likely consequence of anoxia (22)).

Tests wth baclofen gave less compelling results which, however, also suggested no loss of sensitivity of $GABA_B$ receptors.

Thus, it appears that early changes in cellular [Ca] or [ATP] are not of sufficient magnitude to cause a major loss of GABA-sensitivity at a time when IPSPs are rapidly disappearing. The most reasonable explanation is that anoxia interferes very early with either the activity of inhibitory neurons or the release of GABA from their terminals. There is no reason to suspect that inhibitory terminals are more susceptible than excitatory ones. It is therefore probable that, like pyramidal cells, the GABAergic neurons are rapidly, and perhaps even sooner, rendered inexcitable by anoxia and thus fail to respond to synaptic activation. By contrast, axonal excitability in the Schaffer collateral afferent pathway is less sensitive to anoxia (as shown by the relatively well-maintained "afferent volley" response (23)); hence the longer, apparently selective survival of monosynaptic EPSPs.

On the other hand, the slow, and often incomplete post-anoxic recovery of IPSPs suggests a long-term, functional depression, perhaps due to irreversible effects of even brief anoxia. In this respect, GABAergic neurons would differ from the pyramidal cells, whose excitability and other properties (except perhaps for their Ca currents (24)) do not show a comparable, prolonged post-anoxic depression. A selective vulnerability of inhibitory cell function could result in more-or-less permanent hyperexcitability following hypoxia (25). This would be in keeping with some previous findings (26) that inhibitory terminals in the cortex are selectively damaged by hypoxia; though, curiously enough, hippocampal GABAergic *cell bodies* can withstand ischemic episodes that cause massive necrosis of pyramidal cells (27).

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REFERENCES

1. Roberts, E., and Frankel, S. 1950. γ-Aminobutyric acid in brain: its formation from glutamic acid. J. Biol. Chem. 187:55–63.
2. Krnjević, K., and Schwartz, S. 1967. The action of γ-aminobutyric acid on cortical neurones. Exp. Brain Res. 3:320–336.
3. Dreifuss, J. J., Kelly, J. S., and Krnjević, K. 1969. Cortical inhibition and γ-aminobutyric acid. Exp. Brain Res. 9:137–154.
4. Roberts, E. 1976. Immunocytochemistry of the GABA system—a novel approach to an old transmitter. Pages 123–138, *in* Ferrendelli, J. A., McEwen, B. S., and Snyder, S. H. (eds.), Neurotransmitters, Hormones and Receptors: Novel Approaches, Society of Neuroscience, Bethesda, Maryland.
5. Fujiwara, N., Higashi, H., Shimoji, K., and Yoshimura, M. 1987. Effects of hypoxia on rat hippocampal neurones in vitro. J. Physiol. 384:131–151.
6. Leblond, J., and Krnjević, K. 1989. Hypoxic changes in hippocampal neurons. J. Neurophysiol. 62:1–14.
7. Inoue, M., Oomura, Y., Yakushiji, T., and Akaike, N. 1986. Intracellular calcium ions decrease the affinity of the GABA receptor. Nature 324:156–158.
8. Akaike, N., Oyama, Y., and Tokutomi, N. 1988. Inhibition of drug-gated chloride currents by calcium influx in frog sensory neurons. Neuroscience Res. 5:557–562.
9. Stelzer, A., Kay, A. R., and Wong, R. K. S. 1988. $GABA_A$-receptor function in hippocampal cells is maintained by phosphorylation factors. Science 241:339–341.
10. Krnjević, K. 1975. Coupling of neuronal metabolism and electrical activity. Pages 65–78, *in* Ingvar, D. H., and Lassen, N. A. (eds.), Brain Work: The Coupling of Function, Metabolism and Blood Flow in the Brain, Munsgaard, Copenhagen.
11. Krnjević, K., and Xu, Y. 1989. Dantrolene suppresses the hyperpolarization or outward current observed during anoxia in hippocampal neurons. Can. J. Physiol. Pharmacol. 67:1602–1604.
12. Duchen, M. R., Valdeolmillos, M., O'Neill, S. C., and Eisner, D. A. 1990. Effects of metabolic blockade on the regulation of intracellular calcium in dissociated mouse sensory neurones. J. Physiol. 424:411–426.
13. Fox, S., Krnjević, K., Morris, M. E., Puil, E., and Werman, R. 1978. Action of baclofen on mammalian synaptic transmission. Neuroscience 3:495–515.
14. Bormann, J. 1988. Electrophysiology of $GABA_A$ and $GABA_B$ receptor subtypes. TINS 11:112–116.
15. Bowery, N.G., Hill, D.R., and Moratalla, R. 1989. Neurochemistry and autoradiography of $GABA_B$ receptors in mammalian brain: Second-messenger system(s). Pages 159–172, in Barnard, E.A., and Costa, E. (eds.), Allosteric Modulation of Amino Acid Receptors: Therapeutic Implications, Raven Press Ltd., New York.
16. Newberry, N.R., and Nicoll, R.A. 1984. A bicuculline-resistant inhibitory post-synaptic potential in rat hippocampal pyramidal cells in vitro. J. Physiol. 348:239–254.
17. Lambert, N.A., Harrison, N.L., Kerr, D.I.B., Ong, J., Prager, R.H., and Teyler, T.J. 1989. Blockade of the late IPSP in rat CA1 hippocampal neurons by 2-hydroxy-saclofen. Neurosci. Letts. 107:125–128.
18. Gallagher, J.P., Nakamura, J., and Shinnick-Gallagher, P. 1983. The effects of temperature, pH and Cl-pump inhibitors on GABA responses recorded from cat dorsal root ganglia. Brain Res. 267:249–259.
19. Krnjević, K., and Walz, W. 1990. Acidosis and blockade of orthodromic responses caused by anoxia in rat hippocampal slices, at different temperatures. J. Physiol. 422:127–144.
20. Stelzer, A., and Wong, R.K.S. 1989. $GABA_A$ responses in hippocampal neurons are potentiated by glutamate. Nature 337:170–173.
21. Benveniste, H., Drejer, J., Schousboe, A., and Diemer, N.H. 1984. Elevation of the extracellular concentrations of glutamate

and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. J. Neurochem. 43:1369–1374.
22. Krnjević, K. and Xu, Y. (1990). Mechanisms underlying anoxic hyperpolarization of hippocampal neurons. Can. J. Physiol. Pharmacol., 68:1609–1613.
23. Cherubini, E., Ben-Ari, Y., and Krnjević, K. 1989. Anoxia produces smaller changes in synaptic transmission, membrane potential, and input resistance in immature rat hippocampus. J. Neurophysiol. 62:882–895.
24. Krnjević, K., and Leblond, J. 1989. Changes in membrane currents of hippocampal neurons evoked by brief anoxia. J. Neurophysiol. 62:15–30.
25. Ben-Ari, Y., and Cherubini, E. 1988. Brief anoxic episodes induce long-lasting changes in synaptic properties of rat CA3 hippocampal neurons. Neurosci. Letts. 90:273–278.
26. Sloper, J.J., Johnson, P., and Powell, T.P.S. 1980. Selective degeneration of interneurons in the motor cortex of infant monkeys following controlled hypoxia: A possible cause of epilepsy. Brain Res. 198:204–209.
27. Pulsinelli, W.A. 1985. Selective neuronal vulnerability: morphological and molecular characteristics. Prog. Brain Res. 63:29–37.