Neuroscience Letters, 90 (1988) 273–278
Elsevier Scientific Publishers Ireland Ltd.

NSL 05456

Brief anoxic episodes induce long-lasting changes in synaptic properties of rat CA3 hippocampal neurons

Y. Ben-Ari and E. Cherubini

INSERM U-29, Hôpital de Port-Royal, Paris (France)

(Received 24 February 1988; Revised version received 6 April 1988; Accepted 6 April 1988)

Key words: Hippocampal slice; CA3; Anoxia; Synchronized evoked burst

The effects of brief anoxic episodes on rat CA3 hippocampal neurons were studied with intracellular and extracellular techniques in the in vitro slice preparation. After repeated (3–7 times), brief (2–6 min duration each) applications of artificial cerebrospinal fluid (ACSF) saturated with 95% N_2 and 5% CO_2, electrical stimulation of various inputs to CA3 neurons, evoked an excitatory postsynaptic potential (EPSP) followed by an all-or-none burst. This response which persisted for several hours after the last anoxic episode, is reminiscent of the bursts induced by various convulsive agents. Post anoxic bursts are generated by a polysynaptic network which converge on the apical distal segment of CA3 neurons. It is concluded that a repetitive impairement of metabolism produces long lasting changes in the synaptic properties of CA3 neurons.

Interruption of the brain's oxygen supply leads to a rapid loss of consciousness. Despite extensive investigations, there is no adequate explanation for this exquisite sensitivity of the brain to anoxia [10]. A long-lasting anoxic episode produces in hippocampal slices an irreversible loss of synaptic activity [1, 9]. Brief anoxic episodes (a few minutes) produce a reversible blockade of synaptic activity which is associated with a membrane hyperpolarization and fall in input resistance [9, 11, 13]. We report here that repeated brief anoxic episodes, also produce a long-lasting change in the synaptic response of CA3 neurones characterized by the presence of evoked synchronized discharges. These post-anoxic bursts (PAB) which persist for several hours, have several features in common with the synchronized discharges generated by convulsive agents in this region.

Adult male Wistar rats (120–200 g) were killed by cervical dislocation. One hippocampus was quickly removed and transverse slices (500 μm) were cut using a McIlwain tissue chopper. Slices were incubated at room temperature in artificial cerebrospinal fluid (ACSF) for at least 60 min and, when required, one slice was transferred

Correspondence: Y. Ben-Ari, INSERM U-29, Maternité de Port-Royal, 123 Boulevard de Port-Royal, 75014, Paris, France.

to a submerged recording chamber. The slices were superfused at 34°C (2 ml/min) with ACSF of the following composition (in mM): NaCl 126, KCl 3.5, NaH_2PO_4 1.2, $MgCl_2$ 1.3, $CaCl_2$ 2. Glucose 10, $NaHCO_3$ 25, pH 7.3. They were gassed and saturated with 95% O_2 and 5% CO_2. Anoxia was induced by superfusing the slices with ACSF gassed and saturated with 95% N_2 nd 5% CO_2. Field potentials were recorded with glass microelectrodes filled with 2 M NaCl (resistance 5–10 MΩ). Intracellular recordings were made with glass microelectrodes filled with either 4 M potassium acetate or 3 M KCl (DC resistance 40–100 MΩ). Bridge balance was checked repeatedly during the course of the impalement. Electrical stimulation was performed with twisted bipolar NiCr-insulated wires (50 μm, o.d.), using pulses of 10–50 μs duration. Signals were displayed on a digital oscilloscope and recorded on a computer-driven pen recorder.

Post-anoxic bursts: extracellular observations. Superfusion of the slice with a medium saturated with 95% N_2, 5% CO_2 rapidly reduced and blocked the extracellular field excitatory postsynaptic potential (EPSP) evoked in the stratum radiatum (SR) by stimulation of the mossy fibers as well as the somatic population spikes ($n=21$). Recovery was complete within 6–10 min. Repeated anoxic episodes produced an additional striking change. Thus as shown in Fig. 1, the third anoxic episode produced a 75% decrease in amplitude of the field EPSP with full recovery 3–6

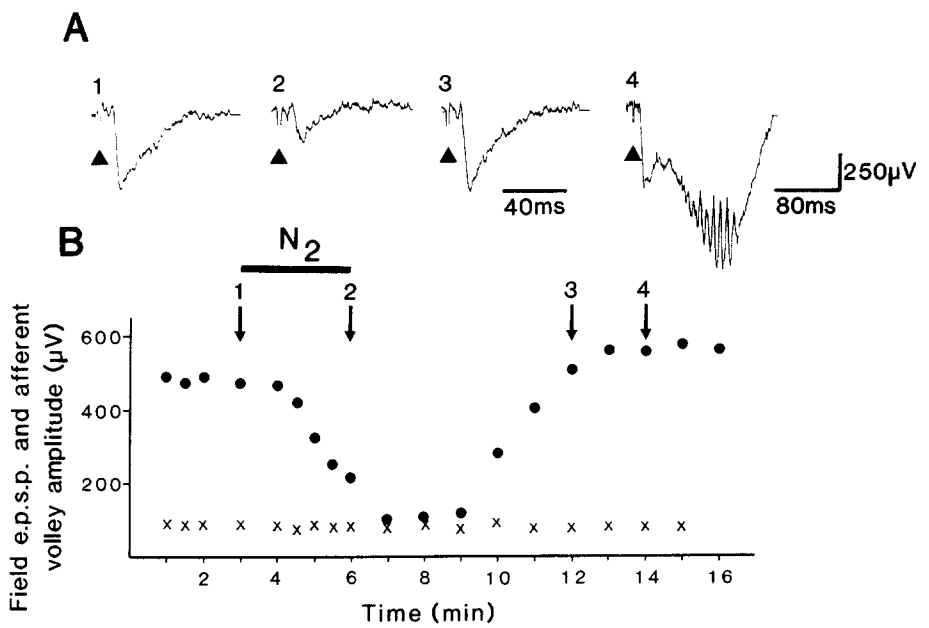

Fig. 1. After 3 anoxic episodes electrical stimulation of the hilus evoked a synchronized discharge. Extracellular recording in stratum radiatum of CA3. A: single displays at points indicated in the graph. B: diagram showing the amplitude of the field EPSP and the afferent volley before, during and after the hypoxic episode. Note in A that before anoxia the stimulation evoked a negative field EPSP preceded by an afferent volley, the former was rapidly blocked by N_2. Eight min after N_2, the stimulation evoked a burst. In this and following figures, the electrical stimulation of the mossy fibers is indicated by dark triangles.

min after reintroducing O_2; this was followed 2 min later by the appearance of evoked bursts. In SR, this consisted of a large negative field EPSP (200–300 ms) and (5–10) positive-going population spikes. PABs were observed in 15 (out of 17) slices, following 3–7 anoxic episodes (2–6 min duration each). They could be evoked for long periods of time (over 3 h).

Post-anoxic bursts: intracellular observations. With intracellular recording the PAB consisted of 5–15 fast action potentials riding on top of a long duration (80–600 ms) giant EPSP (25–55 mV) which was synchronous with the extracellular field EPSP (Fig. 2A). The PAB was observed in every neuron tested ($n=6$). Once the PAB had developed they were evoked by every afferent stimulation. The PAB was readily blocked by an additional anoxic episode, this blockade was associated with a small membrane hyperpolarization during which the stimulation of afferent inputs evoked an action potential riding on a large EPSP (Fig. 2).

Laminar field analysis of post-anoxic bursts. The observation that the PAB was

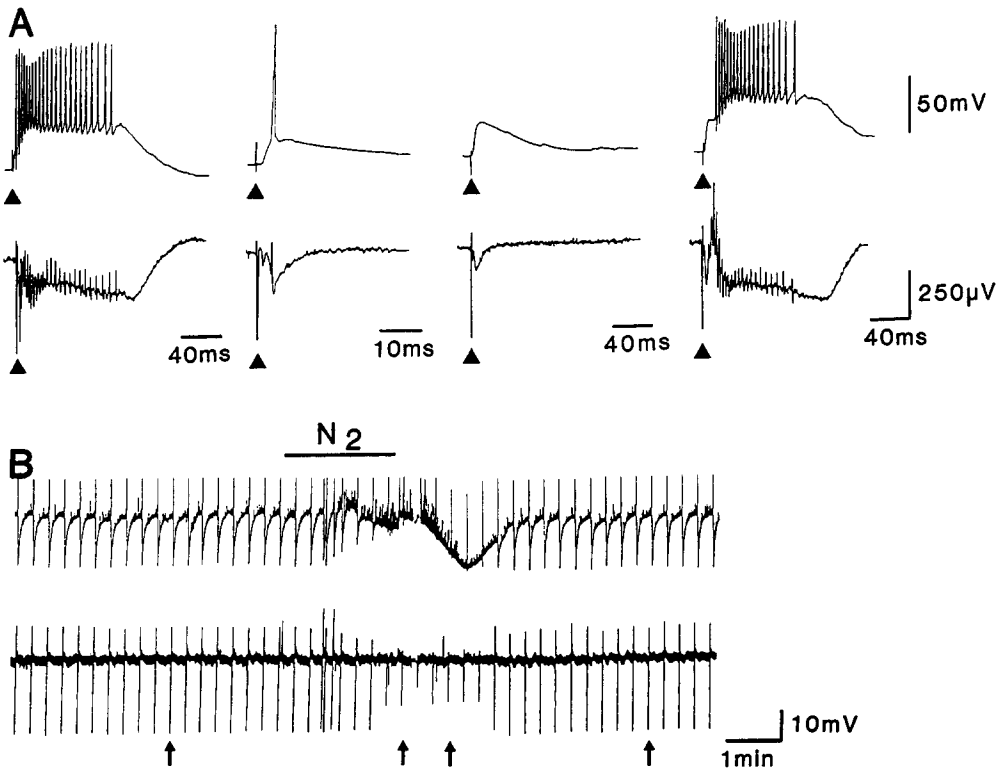

Fig. 2. Intracellular and extracellular recordings to illustrate the effects of anoxic episode on the bursts. Sixth anoxic episode; upper traces intracellular recording, lower traces extracellular recording in stratum radiatium. Note that the burst is composed of 10–20 spikes riding on top of a giant long lasting EPSP. B: continuous chart record. The electrical stimulation of the hilus at a frequency of 0.05 Hz evoked a PAB. Note that during the anoxic episode the burst was first blocked (after less than 1 min), the stimulation only evoked a single spike; a larger EPSP was evoked during the hyperpolarization at the end of the anoxic episode.

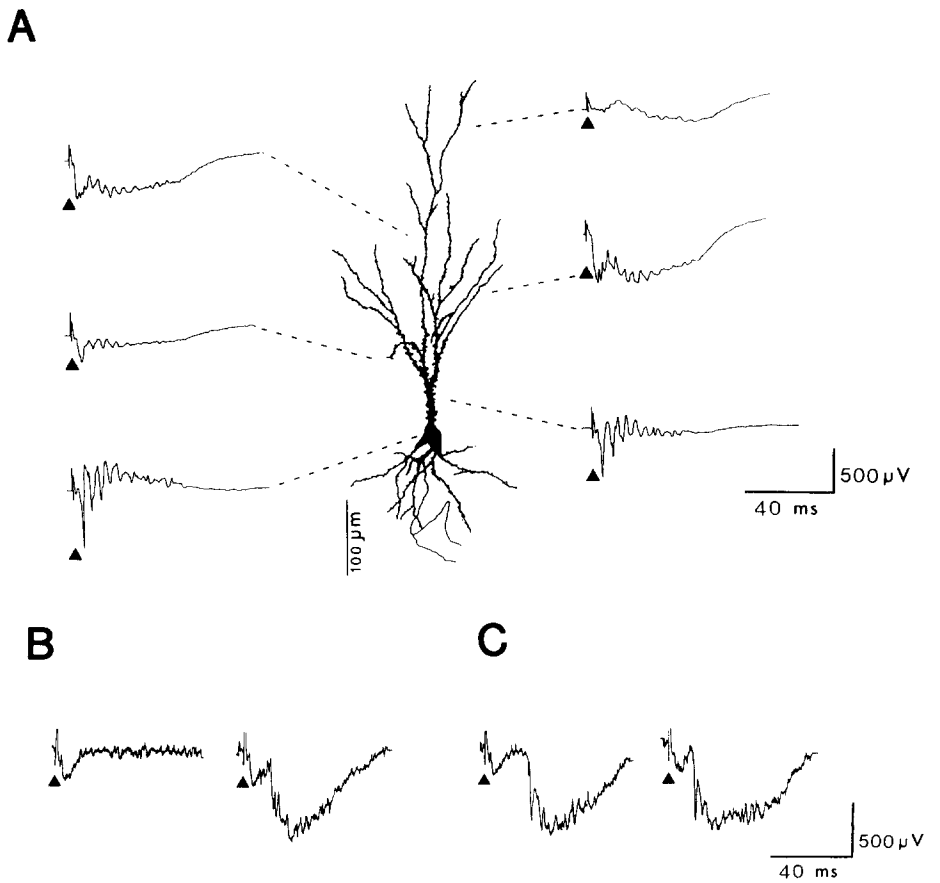

Fig. 3. Properties of extracellular burst. A: laminar profile analysis. An extracellular recording electrode was moved along the dendrites. Note that the PAB was negative at the apical dendrites where they are generated and reversed in the boundary with the mossy fiber region. B: all-or-none feature of the PAB, a stimulus of 7 V produced (left) only an EPSP, a small increase in intensity (9 V) evoked an EPSP followed by a PAB. C: variable latency of the polysynaptic PAB in comparison with the fixed latency of the monosynaptic EPSP.

more susceptible to anoxic episodes than the monosynaptic EPSP (Fig. 2) suggests that it is generated by a polysynaptic network. Other observations confirm this conclusion including the variable latency of the PAB contrasting with the fixed latency of the EPSPs (Fig. 3C) and the all-or-none character of the PAB (Fig. 3B). The PAB was also selectively blocked by elevated concentrations of divalent cations (6 mM Mg^{2+}, 4 mM Ca^{2+}) known to preferentially block polysynaptic responses (not shown, e.g. ref. 6). These observations suggest the PAB are likely generated by a circuit intrinsic to the CA2–CA3 region. To better define its anatomical features, a laminar profile analysis of the field potentials was performed in 3 slices by moving an extracellular electrode along the apical dendrites and pyramidal layer. We found that negative field potentials corresponding to the site of generation of the PAB, were

exclusively recorded in the distal two thirds of stratum radiatum; in contrast, positive fields were recorded in the mossy fiber region and pyramidal layer (Fig. 3).

Since other studies suggest the involvement of NMDA receptor in epileptiform activity [2, 15, 18], we have also tested the effects of D-2-amino-phosphono valerate (20–30 μM, $n=5$ slices) on the PAB and found only a small reduction of the duration of the negative field (not shown).

The present study shows that repeated anoxic episodes produce a long-lasting alteration in synaptic properties of CA3 neurons characterized by the presence of a synchronized discharge evoked by afferent stimuli. Spontaneous network driven bursts similar to the PAB are readily produced in CA3 by a variety of convulsive agents or procedures [2, 4, 8, 12, 16, 17]. Some of these procedures cause in addition to the spontaneous bursts, present during and shortly after the application, a long-lasting change in synaptic response characterized by the presence of evoked synchronized bursts which persist for several hours after reintroducing normal ACSF [2, 4, 8]. Like the PAB the evoked bursts are generated by a polysynaptic network. They have a similar laminar field profile, latency variability, all-or-none character and synchronicity between intracellular and extracellular events [2, 4, 8]. It is therefore likely that the synchronized discharges in CA3 are generated by recurrent excitatory collaterals which interconnect pyramidal neurons [14]. The mechanisms underlying the long lasting changes produced by repeated anoxic episodes or by convulsive agents are presently elusive. Both kainate [5] and anoxia [9] produce a brief–fully reversible reduction of GABAergic inhibition. Such a reduction may participate in the long-lasting change perhaps by means of the activation of *N*-methyl-D-aspartate (NMDA) receptors in keeping with the mechanisms suggested for long-term potentiation and epileptogenesis [7, 15, 18]. The partial reduction of the PAB by NMDA antagonists (see also ref. 3) is consistent with this hypothesis. Whatever the exact mechanism, the present observations are important in suggesting that in contrast to isolated anoxic episodes repeated impairment of oxygen supply produces a long lasting increase in excitability and synchronized epileptiform discharge.

We are grateful to M.S. Guidasci and M.S. Bahurlet for photographic and secretarial assistance respectively. Financial support from the DRET is also acknowledged.

1 Aitken, P.G. and Schiff, S.J., Selective neuronal vulnerability to hypoxia in vitro, Neurosci. Lett., 67 (1986) 92–96.

2 Anderson, W.W., Swartzwelder, H.S. and Wilson, W.A., The NMDA receptor antagonist 2-amino-5-phosphonovalerate blocks stimulus train-induced epileptogenesis but not epileptiform bursting in the rat hippocampal slice, J. Neurophysiol., 57 (1987) 1–21.

3 Aram, J.A. and Lodge, D., Epileptiform activity induced by alkalosis in rat neocortical slices: block by antagonists of N-methyl-D-aspartate, Neurosci. Lett., 83 (1987) 345–350.

4 Ben-Ari, Y., Cherubini, E. and Gho, M., Long-lasting induction of an evoked network burst in rat hippocampal CA3 neurones in vitro, J. Physiol. (Lond.), 388 (1987) 508.

5 Ben-Ari, Y., Gho, M. and Rovira, C., Kainic acid reduces both the baclofen induced outward current and the late inhibitory postsynaptic current in rat hippocampal CA3 neurones in vitro, J. Physiol. (Lond.), 398 (1988) 20.

6 Berry, M.S. and Pentreath, V.W., Criteria for distinguishing between monosynaptic and polysynaptic transmission, Brain Res., 105 (1976) 1–20.
7 Bliss, T.V.P. and Collingridge, G.L., NMDA receptors: their role in long term potentiation, Trends Neurosci., 10 (1987) 238–293.
8 Cherubini, E., Neuman, R., Rovira, C. and Ben-Ari, Y., Epileptogenic properties of the mast cell degranulating peptide in CA3 hippocampal neurones, Brain Res., 445 (1988) 91–100.
9 Fujiwara, N., Higashi, H., Shimoji, K. and Yoshimura, M., Effects of hypoxia on rat hippocampal neurones in vitro, J. Physiol. (Lond.), 384 (1987) 131–151.
10 Haldane, J.S. and Priestley, J.G., Respiration, Yale University Press, New Haven, 1935, pp. 493.
11 Hansen, A.J., Hounsgaard, J. and Jahnsen, J., Anoxia increases potassium conductance in hippocampal nerve cells, Acta Physiol. Scand., 115 (1982) 301–310.
12 Korn, S.J., Giacchino, J.L., Chamberlin, N.L. and Dingledine, R., Epileptiform bursts activity induced by potassium in the hippocampus and its regulation by GABA-mediated inhibition, J. Neurophysiol., 57 (1987) 325–340.
13 Krnjevic, K., Cherubini, E. and Ben-Ari, Y., Effects of anoxia on synaptic transmitter and calcium currents in immature hippocampus, Soc. Neurosci. Abstr., 315 (1987) 9.
14 Miles, R. and Wong, R.K.S., Excitatory synaptic interactions between CA3 neurones in the guinea-pig hippocampus, J. Physiol. (Lond.), 373 (1986) 397–418.
15 Mody, I. and Heinemann, V., NMDA receptors of dentate gyrus granule cells participate in synaptic transmission following kindling, Nature (Lond.), 26 (1987) 701–704.
16 Neuman, R.S., Cherubini, E. and Ben-Ari, Y., Is activation of *N*-methyl-D-aspartate receptor gated channels sufficient to induce long term potentiation, Neurosci. Lett., 80 (1987) 283–288.
17 Rutecki, P.A., Lebeda, F.J. and Johnston, D., Epileptiform activity induced by changes in the extracellular potassium in hippocampus, J. Neurophysiol., 54 (1985) 1363–1374.
18 Stelzer, A., Slater, N.T. and Ten Bruggencate, G., Activation of NMDA receptors blocks GABAergic inhibition in an vitro model of epilepsy, Nature (Lond.), 26 (1987) 698–701.