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# Anoxic changes in dentate granule cells

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In most of the granule cells recorded, by current clamp and single-electrode voltage-clamp (SEVC), only small depolarizations (or inward currents) and minor conductance increases were observed during brief periods of anoxia (2–3 min). Thus, unlike pyramidal cells, granule cell bodies show little sign of K channel activation by anoxia. Post-anoxic hyperpolarizations were also minimal. Moreover, diazoxide (an activator of ATP-sensitive K conductance ($G_{K(ATP)}$)) had no consistent hyperpolarizing action. The depressant effect of diazoxide on anoxic glutamate release from mossy fibres is therefore likely to be mediated by $G_{K(ATP)}$ channels situated on granule cell axons or terminals rather than on the cell bodies.

According to previous studies, granule cells resemble CA1 and CA3 pyramidal cells in their membrane characteristics [7, 10]. But they are far less prone to generate 'burst-type' paroxysmal depolarizations [2, 11], perhaps because their Ca-currents [6] are smaller or because internal free $Ca^{2+}$ is better controlled. This may account for their well-known resistance to noxious effects of anoxia/ischemia, both in vivo [20] and in vitro [1, 15, 18].

Intracellular studies on hippocampal pyramidal cells [12, 14, 16–18] have consistently revealed a predominant hyperpolarizing shift during brief periods of anoxia (though with some indirect depolarizing effects superimposed, especially in CA3 cells [5]), associated with a near 50% reduction in input resistance, as well as a marked post-anoxic phase of hyperpolarization, probably caused by electrogenic Na/K pumping. In the present experiments, we wanted to find out whether granule cells show similar effects of anoxia, and also whether the presence of ATP-sensitive K channels ($G_{K(ATP)}$) could be demonstrated by applications of diazoxide, a selective activator of $G_{K(ATP)}$ [3].

The experiments were done on conventional hippocampal slices (from Wistar rats),

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kept submerged in artificial cerebrospinal fluid (ACSF), at 34°C. Standard current- or voltage-clamp techniques were used to record membrane potential ($V_m$) or current, with single microelectrodes containing 2–4 M KCl, potassium methylsulphate or potassium acetate. Synaptic potentials were evoked by stimulating the perforant pathway; and anoxia was produced by switching to superfusion with ACSF equilibrated with 95% $N_2$–5% $CO_2$, instead of the usual 95% $O_2$–5% $CO_2$.

In 11 granule cells with $V_M$ $-70.9 \pm 3.54$ mV (mean ± S.E.M.), the input resistance ($R_N$) was $73.0 \pm 15.5$ MΩ. Judging by the responses to 200 ms hyperpolarizing current pulses (Figs. 1 and 2), the granule cells had a relatively short membrane time-constant, and they seldom (only 1/11) showed a time-dependent sag of potential, though anomalous rectification was clearly evident in voltage-current plots.

In 30 tests on 9 cells, by far the most common effect of brief anoxia (2–3 min) was a moderate depolarization (Fig. 1), by $8.2 \pm 1.12$ mV (in 19 out of 19 tests in 8 cells),

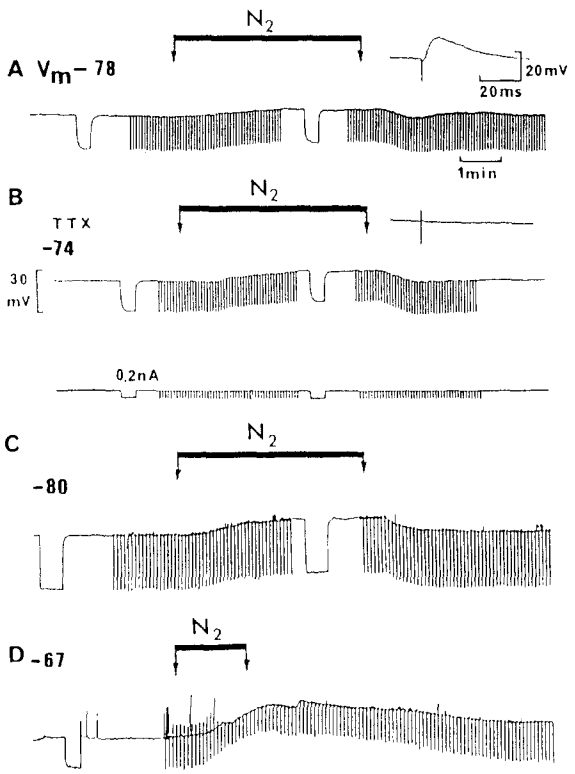


Fig. 1. Anoxia has a TTX-insensitive, mainly depolarizing effect on granule cells. Three cells A, C and D show small depolarizations during superfusion with 95% $N_2$–5% $CO_2$ equilibrated ACSF (labelled $N_2$); current-clamp recordings with potassium acetate electrode for A, and KCl-electrode for C and D. Tests of input resistance were with 200 ms pulses of hyperpolarizing currents: 0.2 nA in A and B, 0.5 nA in C and 0.7 nA in D. Resting membrane potentials ($V_m$) are indicated in mV. B was recorded in same neuron as A, but after complete block of evoked responses by TTX (1 μM): note disappearance of EPSPs initially evoked by perforant path stimulation, illustrated by inset traces at right in A and B. Paper trace was accelerated at intervals to display resistance pulses in greater detail.

accompanied by only a small or no detectable drop in resistance ($-15.5 \pm 3.89\%$). This depolarization was not reduced or eliminated by tetrodotoxin (TTX) (cf. Fig. 1A, B) — unlike the depolarizations seen in CA3 pyramidal cells, which appear to be mainly caused by glutamate release from afferent terminals [4, 5]. In 3 tests during single-electrode voltage-clamp (SEVC) (at $V_H$ $-70$ mV), there were corresponding small inward current shifts ($-0.11 \pm 0.045$ nA) and increases in conductance. Only in one neuron did anoxia have a clear hyperpolarizing effect ($-7.8 \pm 2.68$ mV, for $n=3$). Post-anoxic hyperpolarizations were also relatively inconspicuous in granule cells ($-1.2 \pm 0.68$ mV).

In experiments on CA3 pyramidal cells [4, 5] (and in preparation), anoxic depolarizing effects mediated indirectly could be much reduced by agents that are known to activate ATP-sensitive K channels (diazoxide [3]), galanin [8] and somatostatin [9]); and conversely they were enhanced by glibenclamide (GLIB) [19], an antagonist of these channels [3]. Because most of these agents had no detectable post-synaptic actions, it was postulated that the $G_{K(ATP)}$ channels must be present on presynaptic elements.

The possibility that $G_{K(ATP)}$ channels might occur throughout presynaptic neurons, including the granule cell *bodies*, was therefore examined, mainly by applications of diazoxide (Unicet, SA, France). At a concentration of 0.87 mM in the superfusing ACSF, 6 tests of diazoxide, for 3–7 min, produced no consistent change either in $V_M$ ($-0.30 \pm 1.51$ mV), or $R_N$ ($-0.96 \pm 1.84\%$). For example, the neuron illustrated in Fig. 2 showed only a minimal hyperpolarization and no reduction in $R_N$ when diazoxide was applied (A); and GLIB had no detectable effect (B). In one recording by SEVC, there was also no significant change in holding current or con-

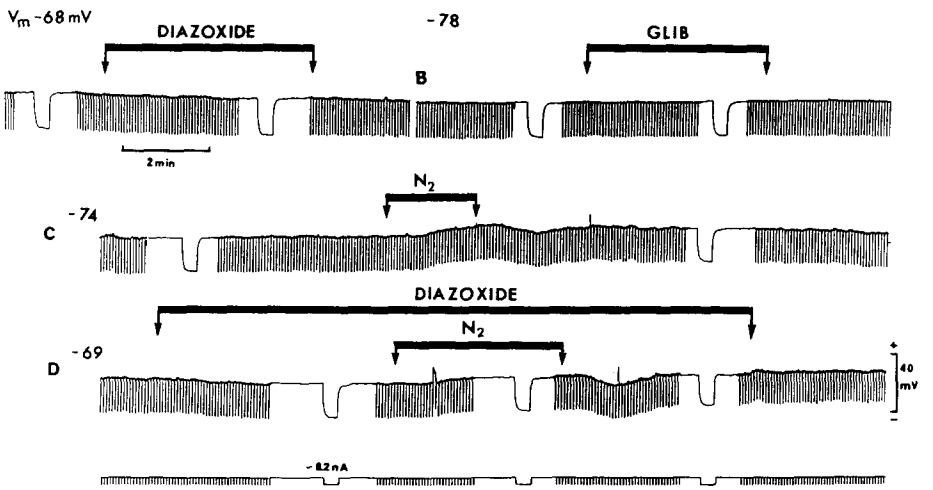


Fig. 2. ATP-sensitive K channels appear to be absent in granule cell bodies. A, B: there are only minimal changes in $V_m$ and $R_N$ when diazoxide (0.87 mM) or glibenclamide (10 $\mu$M) was superfused. Moreover, depolarizing effect of anoxia — shown in C — was unaffected by concurrent application of diazoxide (0.87 mM), as illustrated in D. Identical 200 ms hyperpolarization current pulses (shown in lower trace, in D) were applied throughout; all recordings from same granule cell, with a potassium acetate microelectrode.

ductance. Furthermore, diazoxide did not eliminate the depolarizing effects of anoxia (cf. traces C and D in Fig. 2).

Thus in constrast to CA1 and CA3 pyramidal cells, most granule cells do not show marked hyperpolarizing, $G_K$-mediated effects of anoxia. If $G_K$ is activated by increased $[Ca^{2+}]_i$ [16, 17], the lack of $G_K$ activation may indicate a paucity of $G_{K(Ca)}$ channels; or, perhaps more likely, smaller Ca fluxes or better control of $[Ca]_i$. This would be in keeping with the granule cells' lower susceptibility to anoxic damage, which is probably caused by an excessive rise of $[Ca]_i$ during anoxia [15, 21]. On the other hand, the minor depolarizations and resistance changes seen during anoxia could be mainly due to small increases in $[K]_o$ — such as occur in all parts of the brain during anoxia [13], presumably owing to diminished Na/K pumping — as well as enhanced transmitter release. In spite of the presence of glibenclamide binding throughout the dentate gyrus [19], the lack of any consistent effect of diazoxide on $V_m$ and $R_N$ suggests that there are only few functional $G_{K(ATP)}$ channels on granule cell bodies. These observations support the proposal that the $G_{K(ATP)}$ channels which appear to modulate anoxic glutamate release on CA3 pyramidal neurons are mainly concentrated on mossy fiber terminals [4, 5].


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