Effect of hypoxia on evoked responses in cerebellar Purkinje cells of the *mdx* mouse

D.K. Chelvanayagan, S.I. Head and J.W. Morley, Department of Physiology, University of New South Wales, Sydney, NSW 2052, Australia .

In Duchenne muscular dystrophy (DMD) the absence of the X-linked recessive gene product dystrophin is associated with progressive muscle wasting and in many cases a cognitive impairment. The distribution of dystrophin is not restricted to muscle, isoforms are found in certain cells in the brain, including the hippocampus and cerebellar Purkinje cells. In this study we investigated the effects of hypoxic stress on synaptic transmission in dystrophin-deficient cerebellar Purkinje cells. We used the *mdx* mouse, an established animal model of DMD, to determine the effect of the absence of dystrophin on synaptic transmission. We recorded intracellularly from Purkinje cells (PCs) in cerebellar slices from the *mdx* mouse and litter mate controls in normal and hypoxic conditions. Mice aged 7-12 weeks were deeply anaesthetized with halothane. The cerebellum was removed rapidly and placed in ice cold artificial cerebrospinal fluid (aCSF) that was gassed continuously with carbogen (95% O_2, 5%CO_2). Parasaggital slices were cut at 250 μm, incubated at 34^{o}C for 40-60 min and then maintained at room temperature for at least 1 h before use. Slices were placed in a chamber on a microscope stage superfused with ACSF, at a rate of 1.2 ml/min at room temperature. Recordings were made using standard glass recording microelectrodes (50 MΩ, 3M KCl). A stimulating electrode was placed nearby (< 250 μm) in the molecular layer to evoke excitatory synaptic potentials (EPSP) of no more than 7mV once every 30s. After a stable recording was obtained for about 10 min, the PC was challenged with hypoxic episodes by changing from a normal aCSF gassed with carbogen to a solution gassed with (95% N_2, 5% CO_2) with or without glucose, for the latter equimolar sucrose was substituted for glucose. In the first series of hypoxia studies we used two 12 minute periods of hypoxic challenge, with 20 minute recovery period between each challenge. The first period was in the presence of glucose (HG), the second in the presence of sucrose (HS). EPSPs largely persisted in amplitude and initial rising slope until abolished from 9-14 min following the start of the period of hypoxia. The EPSPs of some PCs failed to abolish during the initial hypoxic challenge (1 of 6 controls and 4 of 13 *mdx*), however, EPSPs were abolished in all cells following the second 12 min exposure (HS). In the final recovery phase, the level of recovery of the amplitude and initial slope of the EPSPs was significantly greater ($p = 0.03$) in the *mdx* PCs ($n = 9$) compared to littermate control PC ($n = 5$). In the second series of studies we used a longer period of hypoxia (> 30 min), and in both *mdx* and control cells the Vm usually showed a shallow depolarizing decline reaching an average maximal 66 mV, that abruptly reversed into a short lived hyperpolarization followed by a rapid anoxic depolarization (AD). There was no significant difference between the *mdx* ($n = 12$) and control ($n = 9$) in the time it took to reach AD. However, in a subset of PCs that hyperpolarized beyond their normal resting level, 3 of 6 control and 6 of 9 mdx cells, a significantly stronger hyperpolarization was apparent in control cells compared with mdx cells ($p < 0.05$). If the long hypoxic challenge was arrested soon after the AD began, PCs would usually recover fully. At that point glibenclamide was added to the normal aCSF, whereupon a second challenge (gHS) was maintained until AD. As before EPSPs were abolished during gHS, however on recovery EPSP ../72P/amplitude and initial slope were substantially greater than pre-hypoxia levels in the *mdx* compared to control cells, implicating ATP sensitive K^+ channels. Although glibenclamide reduced shunting of the membrane resistance at AD, hyperpolarization had still occurred indicating involvement of other K^+ channels, most likely Ca^{2+} sensitive. While the hypersensitivity of dystrophin positive cells to global ischemia reported in the hippocampus, cortex and cerebellum (Mehler *et al.*, 1992; Godfriend *et al.*, 2000) was not apparent in our cerebellar slice preparation, our data are consistent with findings of others (Godfriend *et al.*, 2000) in that we show a significant difference in post-hypoxic recovery of synaptic transmission in the presence of low glucose in *mdx* compared to controls PCs. These data suggest that dystrophin-deficient Purkinje cells may be more resistant to the damage induced by repeated or prolonged periods of hypoxia.

Mehler MF, Haas KZ, Kessler JA & Stanton PK. (1992) *Proceedings of the National Academy of Science USA.*, **89:** 2461-5.

Godfraind J-M, Tekkok SB & Krnjevic K. (2000) *Journal of Cerebral Blood Flow & Metabolism*, **20:** 145-52.