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Baroreflex-autonomic control of regional coronary blood flow conductance in awake sheep

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We have previously shown in the awake dog that integrated baroreflex control of autonomic nerves investing the coronary vessels varies between coronary territories during acute, then steady-state changes in arterial pressure (Quail *et al.*, 1996). It is not known how such mechanisms vary in other species. Confirmation of like mechanisms would offer understanding of reflex coronary autonomic function in other mammals including man. Reflex coronary mechanisms in man are unknown. An awake sheep model was developed for this investigation (Hamut *et al.*, 2006). Merino ewes (n = 5) were prepared under general anaesthesia, *i.e.*, i.v. propofol 5mg/kg, followed by inhaled 2.5-3.5% isoflurane in oxygen. Pulsed Doppler flow probes were implanted on the right, circumflex, and anterior descending coronary arteries. Pacemaker leads were attached to the left atrial appendage and right ventricle. An external vascular occluder was placed around the descending thoracic aorta to control changes in aortic pressure (AoP), and central aortic and venous catheters were positioned in superficial cervical artery and vein to measure pressures (Bishop *et al.*, 2003). The sheep recovered for at least one week. Data were collected under conditions of rest and raised AoP at a constant pacing rate of 150/min, with and without combined cholinoceptor, β1- and β2-adrenoceptor, and α1- and α2-adrenoceptor control (''Total autonomic block'', (TAB)). This was achieved using combined i.v. methscopolamine bromide 270 microgram/kg, propranolol 1 mg/kg, and phentolamine 0.4 mg/kg statum, 1mg/min continuous infusion.

On raising aortic pressure serving all 3 coronary beds in the autonomic intact state, AoP rose by 14, 28 and 32 mmHg at 4s, 8s (immediate reflex period) and 25s (steady-state reflex period), respectively. Changes in coronary conductance varied between time intervals in each bed, and also between beds at specific times. In the circumflex bed at 4 and 8s, conductance rose significantly to 109% ($p < 0.001$) and 104% ($p < 0.05$) of resting control, respectively, but was not changed at 25s. By contrast, while at 4s conductance rose in anterior descending bed to 104% ($p < 0.01$), it did not change in the right coronary bed. In the right bed at 8 and 25s, coronary conductance fell substantially to 87% ($p < 0.01$) and 72% ($p < 0.001$), respectively. In the anterior descending bed at 8 and 25s, conductance fell to 92% ($p < 0.001$) and 91% ($p < 0.001$), respectively. In TAB, the responses in the circumflex bed were strongly modified at 4 and 8s, but not in the steady-state. At 4s, the rise in circumflex conductance was halved, and at 8s reversed, to a significant vasoconstriction to 92% ($p < 0.001$). During TAB, there were minor changes in the response of the anterior descending and right coronary beds.

Therefore, reflex neural vasodilator events dominate myogenic and metabolic local responses mainly in the circumflex bed during the immediate rise in AoP, but do not in the steady-state of maintained AoP. The data suggests neural controls facilitate circumflex coronary flow as an immediate response to rapid left ventricular loading. The effect on circumflex flow is to raise flow to a level (136% of resting) above that observed in the absence of neural control (124%). These effects resemble those in the awake dog. However, in the dog immediate baroreflex dilator effects in the right coronary circulation exist at elevated ventricular rates (White, 1998), and these are absent in sheep. The difference in the right coronary circulation may reflect in sheep a more substantial right coronary vascular bed serving the septal and more distal myocardium relative to the base of the heart, where, *e.g.*, vagal innervation, is less extensive. The response of the anterior descending bed appears similar between the species, and is largely without reflex vasodilator effects.

It is concluded that baroreflex-autonomic control of coronary conductance in the awake sheep varies regionally as it does in the awake dog. The vasodilator events in the circumflex and vasoconstrictor events in the anterior descending beds resemble those in the dog, but in the sheep they are vasoconstrictor in the right coronary circulation.

Quail AW, Cottee DBF, White SW, Porges WL & Hennessy EJ. (1996) *Clinical and Experimental Pharmacology and Physiology*, **23:** 866-73.

Hamut M, Quail A, Cottee D, Seah P, Blake R, White S, McLeod D & Bishop R. (2006) *FASEB Journal*, **20:** A1398.

Bishop R, McLeod D, McIlveen S, Blake R, Gunther R, Davis J, Talken L, Cottee D, Quail A, Parsons G & White S. (2003) *Archives of Physiology and Biochemistry*, **111:** 313-4.

White SW. (1998) *Cardiologia*, **43:** 559-70.

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Evolutionary aspects of neural control of coronary blood flow

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An evolutionary perspective of the autonomic nerves and their many transmitters innervating the coronary circulation is reasonably well described, but the functional significance of this anatomy is poorly understood. This is a fertile area for investigation, given the poor oxygen reserve for defence normally in the myocardium, and the morbid impact of obstructive coronary disease. However, among mammalian species including man, Gregg & Fisher (1963) point out that the course and distribution of the major coronary arteries is remarkably similar. Intergroup differences are less pronounced than intragroup variations. Nevertheless, despite this anatomical knowledge, there is little systematic comparative data concerning functional neural control of coronary blood flow among mammalian species, and what exists implies that neural control of the coronary circulation differs between species. The enigma is manifest by the variable findings with acetylcholine *e.g.*, Kalsner (1985) in his review, concluded that coronary resistance vessels from most species constrict in response to acetylcholine, and that the dog was the only species where coronary vessels dilate. Also, the diving seal constricts the circumflex bed possibly by atropine sensitive pathways (Elsner *et al.*, 1985). In the midst of this uncertainty, one would think a successful design in evolutionary terms for neural coronary blood flow control in one mammalian species would be present in others. Finally, there is still a general assumption, probably erroneous (see below, Quail *et al.*, 1996; White, 1998), that autonomic control of the different myocardial regions supplied by the right, circumflex and anterior descending coronary arteries, is uniform in effect.

Recently, we noted in the awake dog that baroreflex regulation of coronary blood flow and conductance varies between the left coronary beds when aortic pressure rises (Quail *et al.*, 1996; White, 1998). Moreover, we were surprised to find that while baroreflex augmentation of coronary conductance appeared small in the face of loading the left ventricle, the quantitative neural effects were quite large when unmasked by the appearance of a strong constrictor response to the same stimulus following pharmacological autonomic blockade of cholinoceptors, α_1-, α_2-adrenoceptors, and β_1-, β_2-adrenoceptors ("total autonomic block", TAB). In other words, the underlying myogenic constrictor response of the coronary vessels to an aortic pressure rise appears to dominate dilator metabolic effects, and in the absence of baroreflex-induced flow augmentation, the rise in blood flow is less than when the autonomic receptor mechanisms are intact. This may be important given the poor oxygen reserve in the coronary beds. Therefore, in the process of defence against multiple environmental stimuli normally confronting mammals, important reflex dilator neural factors prevail over constrictor factors. This is an interaction effect, largely hidden from experimental gaze in bench studies, and in less sensitive physiological models where anaesthetic agents themselves variably block pathways actually being sought.

In order to investigate baroreflex regulation among mammalian species, we recently compared the responses of awake dogs, with those in awake sheep (see Hamut *et al.*, this issue). Interestingly, the results show that in the regions supplied by the circumflex and anterior descending coronary arteries, the baroreflex pattern of effect across the beds in sheep is qualitatively similar to that of the dog. However, the quantitative effects in sheep are less than in the dog. The main similarity between species is the neural augmentation flow effect in the circumflex bed when aortic pressure rises. In the sheep this amounts to 136% of control at 8s in the intact animal, but is significantly less at 124% following TAB. These effects are relatively less in the anterior descending bed, and not apparent in the right coronary bed. The quantitative role of the different receptor classifications and their interactions within the neural control 'TAB' examined in these studies, and the role of other (residual) neural mechanisms, remains to be elucidated. It is concluded that the baroreflex patterns of neural control in different left coronary beds are qualitatively similar in dogs and sheep. This suggests that a common evolutionary pattern of neural control has emerged, within mammalian species.

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White SW. (1998) *Cardiologia*, **43:** 559-70.

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Integrated autonomic control of the bronchial circulation and 3rd generation airway dimensions during exercise in awake sheep

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Background. The onset of strenuous exercise induces increases in parasympathetic cholinergic bronchovascular constrictor activity, and causes bronchoconstriction of the left main bronchus in concert with sympathetic adrenergic constriction of systemic vascular beds (Quail *et al.*, 2007). The aim of this study was to define changes in bronchial blood flow (Q_{br}) and 3rd generation airway dimensions during moderate exercise, and to analyse the role of vagal and sympathetic nerves.

Methods. Eight ewes (35-50 kg) underwent left thoracotomy during general anaesthesia (*i.e.*, i.v. propofol 5 mg•kg^{-1}, followed by inhaled 2.5-3.5% isoflurane in oxygen) and were instrumented with pulsed-Doppler flow probes mounted on the bronchial artery, and transit time, plus single crystal sonomicrometer transducers mounted on the 3rd generation lingular lobe bronchus. These measured continuously Q_{br}, bronchial hemi-circumference ($CIRC_{br}$) and wall thickness (WALL TH_{br}). Aortic pressure (P_a) and central venous pressure (P_v) catheters placed in the superficial cervical artery and vein. Sheep were recovered for at least seven days prior to experimentation. In Protocol 1 (P1), eight sheep ran duplicate protocols on a horizontal treadmill at constant speed of 2.2 mph for 2 min, 10 min recovery, for analysis of the effects of moderate exercise on changes in Q_{br} and airway dimensions. In P2, five sheep underwent moderate exercise both before (autonomic-intact, INT) and after α_1- α_2-adrenoceptor blockade (α-BL) with i.v. phentolamine. In P3, eight sheep underwent moderate exercise before and after β-adrenoceptor blockade (β-BL) with i.v. propranolol, and in P4, before and after cholinoceptor blockade (chol-BL) with i.v. methscopolamine.

Results. In P1, during moderate exercise P_a and heart rate (HR) rose significantly. $CIRC_{br}$ fell immediately to 96% ($p < 0.001$) of resting levels with the onset of exercise, and remained at similar levels throughout exercise, before returning to resting levels at the cessation of exercise. With exercise onset, WALL TH_{br} rose to 102% ($p < 0.05$) of resting levels, remained at this level throughout exercise, before returning to resting levels during recovery. Q_{br} and blood flow conductance (C_{br}) fell immediately with exercise to 91% and 84% ($p < 0.05$) of resting control levels, respectively. Q_{br} and C_{br} returned slowly towards resting levels during exercise, and fell again briefly in recovery to 84% ($p < 0.05$) and 80% ($p < 0.01$) respectively, before returning to pre-exercise levels in recovery. In P2, moderate exercise caused a fall in $CIRC_{br}$ and rise in WALL TH_{br} in both the INT and α-BL states. The fall in Q_{br} with exercise was seen in both the INT and α-BL states, but the immediate fall in C_{br} with exercise onset in the INT state was not seen with exercise following α-BL. In P3, the response of airway dimensions following β-BL was similar to the INT response to exercise. Following β-BL, there was a significant fall in Q_{br} and C_{br} at the onset of exercise, and at 2 min into exercise, the latter fall not seen during INT exercise. In P4, following chol-BL, the fall in $CIRC_{br}$ (to 98% of resting levels, $p < 0.01$) with exercise onset, was smaller than that seen during INT exercise (95%, $p < 0.001$). Also, $CIRC_{br}$ in the chol-BL state returned towards control levels at 1 min into exercise, whereas it remained below resting levels for the duration of INT exercise. Following chol-BL, the changes in WALL TH_{br} with exercise were similar to those seen during INT exercise. The fall in Q_{br} and C_{br} with moderate exercise was seen in both the chol-BL and INT states, but the brief post-exercise fall in C_{br}, present in the INT state, was no longer present in the chol-BL state.

Conclusions. With the onset of moderate exercise, the reduction in airway hemi-circumference and increase in bronchial wall thickness indicate a fall in cross-sectional area, and thus an increase in airflow resistance at the level of the third generation bronchus. This exercise-induced bronchoconstriction occurs concurrently with vasoconstriction of the bronchovascular bed. The primary autonomic effect responsible for lower airway constriction during exercise is mediated through cholinergic pathways. The primary autonomic effects responsible for bronchial vascular constriction during moderate exercise are mediated by integrated α-adrenergic and cholinergic pathways. During moderate exercise, differential CNS resetting of autonomic activity simultaneously increases: i) sympathetic excitability to airway blood vessels to cause a mild fall in bronchial blood flow conductance; and ii) parasympathetic, vagal excitability to the bronchial wall to cause airway constriction, and bronchovascular constriction to complement sympathetic vasoconstrictor effects.

Quail A, McIlveen S, Bishop R, McLeod D, Gunther R, Davis J, Talken L, Cottee D, Parsons G. & White, S. (2007) *Pulmonary Pharmacology and Therapeutics*, **20:** 190-9.

Controversy: exercise-induced pulmonary haemorrhage in the horse

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Exercise-induced pulmonary haemorrhage (EIPH) is of concern world-wide due to high numbers of thoroughbred horses which bleed. Once detected, horses are banned from racing due to potential collapse on the track and the risk to jockeys and other starters. In Australia the initial ban is 3 months; another bleed incurs a life-time ban. The cost to the racing industry of this rejection is spectacular. In Australia, the issue is the subject of Government Reports. The financial input estimate to the industry is US$ 6.24B. In 2006 it was estimated that approximately 17,280 horses bled with a potential life-time ban and output cost of US$ 70.4M. However, the postulated mechanisms and solutions are dependent on experimentally testable postulates, which are highly controversial and expensive to test. EIPH occurs in any working horse, and in other species at high work levels, including Olympic man.

Current theories (West, 2003) focus on extreme pressures on the heart and pulmonary blood vessels during, and at the end of an event. Limited data suggests elevation of mean aortic pressure to 240 mmHg, mean pulmonary artery pressure to 120 mmHg, an estimated pulmonary capillary pressure of 70 mm Hg, and left atrial pressure to 70 mm Hg. These pressures may rupture the ‘thin but strong’ alveolar membrane made of epithelium, collagen IV, and endothelium. By contrast, Manohar (1992) argues that the bronchial capillary bed may rupture.

We favour the hypothesis that the bronchial capillary bed is more vulnerable to the physical forces than the alveolar circulation, because, i) if the intravascular pressures are as documented, it is likely the systemic bronchial capillary is subject to higher pressures than the pulmonary capillary; ii) the protection of pulmonary capillary pressure afforded by vasoconstriction evoked by excitation-resetting of CNS vagal activity during exercise (Quail *et al.*, 2007), may be withdrawn by thermoregulatory inhibition; iii) the high pressure in the bronchial circulation is also beyond any autoregulatory control of resistance vessels. Capillary pressures will thus rise due to high distending pressures invoked by the high systemic (upstream) mean pressure and high pulmonary (downstream) mean pressure, irrespective of drainage site. It is inescapable that bronchial capillary pressures must exceed pulmonary capillary pressure. These vessels lack the ‘thin but strong’ structure of the alveolar membrane, and will thus constitute the primary site of EIPH.

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Manohar M. (1992) *American Journal of Veterinary Research*, **53:** 925-9.
Quail A, McIlveen S, Bishop R, McLeod D, Gunther R, Davis J, Talken L, Cottee D, Parsons G & White S. (2007) *Pulmonary Pharmacology and Therapeutics*, **20:** 190-9.

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The effects of maternal renal insufficiency on glomerular haemodynamics and tubuloglomerular feedback in the ovine fetus

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Maternal renal insufficiency, caused by subtotal nephrectomy (STNx) of the ewe prior to mating, resulted in a number of fetal effects suggestive of fetal volume expansion (Gibson *et al*., 2007). Since we have previously shown that tubuloglomerular feedback (TGF) is operative in fetal life, and that volume expansion causes a decrease in fetal TGF sensitivity (measured as an increase in the turning point (TP) of the fetal TGF curve; Brown *et al*. (2005)), we hypothesized that TP would be increased in fetuses of STNx ewes.

At least two months prior to mating, STNx was carried out under general anaesthesia (1 g sodium thiopentone i.v. followed by 1-3% halothane in oxygen). The right kidney was removed and a branch of the left renal artery (supplying at least one third of the kidney) was ligated. At 137-141 days (term = 150 days) fetuses of both STNx ewes (STNxF) and intact ewes (IntF) were prepared for either renal micropuncture or blood flow measurements. Ewes were anaesthetised with i.v. thiopentone and maintained with 2-4% isoflurane in 100% oxygen via a ventilator. Maternal jugular and carotid vessels were catheterized. After exposure of the uterus via a midline abdominal incision, the fetal lower body was exteriorized and catheters inserted into both tarsal veins, a femoral artery and suprapubically into the bladder. Fetuses were delivered into a water bath (39°C) with care taken to maintain umbilicoplacental blood flow. Vecuronium (0.1 mg/kg i.v.) was administered to the ewe and fetus as needed. In eight IntF and seven STNxF, the left kidney was prepared for micropuncture. In six IntF and eight STNxF, transonics flow probes were positioned around the abdominal aorta and left renal artery for measurement of blood flows. TGF activity was assessed by measuring proximal tubular stop-flow pressure (P_{SF}) in response to various perfusion rates of the loop of Henle.

Although renal blood flow was similar in STNxF and IntF, glomerular filtration rate (GFR, measured as clearance of endogenous creatinine) was higher in STNxF (7.2 ± 0.7 *vs.* 4.6 ± 0.5 (SE) ml/min, $p < 0.01$). Despite this higher GFR, net filtration pressure (NFP) was lower in STNxF than IntF (19.2 ± 0.4 *vs.* 24.4 ± 0.9 mmHg $p < 0.001$). Consequently the calculated filtration coefficient was higher in STNxF ($p < 0.001$). While the maximal change in P_{SF} (ΔP_{SF}; a measure of TGF reactivity) was reduced in STNxF (5.8 ± 0.2 *vs.* 7.1 ± 0.4 mmHg, $p < 0.05$), the turning point (TP) was similar in the two groups (15.0 ± 1.4 *vs.* 16.0 ± 1.3 nl/min).

Contrary to our prediction, TP was not increased in STNxF. However, these STNxF were ~ 10 days older than those we had previously studied (aged 126-128 days). Furthermore, a number of the findings that were suggestive of volume expansion in the younger STNxF (*e.g.*, reduced haematocrit and depressed fractional reabsorption of sodium by the proximal tubule) were not present in this older cohort. Since younger STNxF had GFRs that were similar to IntF (3.6 ± 0.6 *vs.* 2.9 ± 0.5 ml/min, Gibson *et al.*, 2007), the higher GFR in the older STNxF compared to IntF, may indicate that with age, STNxF have managed to adapt their renal function to compensate for the altered maternal fluid and electrolyte balance.

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Spontaneous Ca and electrical signals in the renal pelvis that drive pelviureteric peristalsis

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Electrical rhythmicity near the base of the papilla in the renal pelvis provides the fundamental drive for the peristaltic contractions that propel urine from the kidney to the bladder for storage before micturition. The histological identification of atypical smooth muscle cells (ASMCs) within these proximal regions of the renal pelvis has often been used to suggest that these cells are the pacemaker cells driving pelviureteric peristalsis. We have separately recorded the electrical activity and associated Ca^{2+} transients in ASMCs using intracellular microelectrodes and the fluorescent Ca^{2+} indicator fluo-4. These spontaneous activities were compared with the equivalent activity in the typical smooth muscle cell (TSMC) wall responsible for the peristaltic contractions. Nifedipine (1-10 μM)-sensitive action potentials and Ca^{2+} waves propagated through the TSMC muscle layer at a velocity of 1 mm•s^{-1} and a frequency of 5-10 min^{-1}. High frequency (>10-40 min^{-1}) spontaneous transient depolarizations (STDs) and Ca^{2+} transients in short spindle shaped cells were resistant to nifedipine blockade and did not propagate over distances >50 mm. STDs and Ca^{2+} transients in ASMCs were blocked in Ca^{2+} free solutions and upon blockade of the Ca^{2+}ATPase pump with cyclopiazonic acid (CPA). STD and Ca^{2+} transients were abolished upon blockade of IP_3 dependent Ca^{2+} store release, but only partially reduced upon blockade of ryanodine receptor Ca^{2+} release channels. STDs were little affected by agents that block pacemaker currents in cultured intestinal interstitial cells of Cajal, La^{3+} and Gd^{3+}, the Cl^- channel blocker, DIDS or upon removal of 85% of the extracellular Cl^-. We speculate that ASMCs act as the primary pacemaker signal in the renal pelvis by generating Ca^{2+} transients and cation selective STDs. In addition, ASMCs appear to be providing point sources of excitation to the TSMC layer.

Mapping action potential initiation sites in corneal cold receptors

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Very little is known about the process of sensory activation of thinly myelinated (Aδ) and unmyelinated (C) sensory neurones that supply the majority of tissues in the body. Both these types of sensory neurone form highly branched nerve terminal arbours with many naked nerve endings. In the cornea, which is exclusively innervated by Aδ- and C-fibre sensory neurones, spike shape analysis and pharmacological blockade of Na^+ channels indicates that the most terminal region of the axon branches is able to support regenerative action potentials (APs) (Carr *et al.*, 2002). This finding leaves open the possibility that APs may be initiated in the most distal portions of the axon branches in these neurones. In the present study we used extracellular recording at the surface of the guinea-pig cornea to record AP activity directly from the 'naked' nerve terminals of cold sensitive neurones (Brock *et al.*, 1998). Briefly, the eyes were dissected from guinea pigs killed by an overdose of pentobarbitone sodium (100 mg/kg). The eyes were mounted in a recording chamber and the optic nerve and associated ciliary nerves drawn into a suction-stimulating electrode. Spontaneous and electrically evoked nerve terminal impulses (NTIs) were recorded with glass electrodes (~50 μm tip diameter) applied to the epithelial surface of the cornea. Cold receptors have an ongoing discharge of APs that is decreased and increased by heating and cooling respectively. Temporal mapping by collision of ongoing APs with electrically evoked antidromic APs, reveal that in most receptors spontaneous impulses are initiated at a site located close to site of recording at the nerve terminal. Analysis of NTI shape was used to investigate changes in the configuration of these signals when they were produced by APs initiated close to the site of recording. Most NTIs are diphasic (positive-negative), with the initial positive phase reflecting the discharge of membrane capacitance as the action potential invades the nerve terminal. Therefore most NTIs are generated by action potentials that are initiated at a point that is electrotonically distant from the nerve ending and that propagate antidromically to site of recording. However, in a small proportion of recordings, ongoing NTIs with an initial negative component due to local influx of Na^+ were recorded, indicating APs initiated at a site electrotonically very close to the site of recording. The results suggest that the sensory signal transduction and regenerative process producing APs can lie in parallel within the nerve terminal.

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Carr RW, Pianova S & Brock JA.(2002) *Journal of General Physiology*, **120:** 395-405

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

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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°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.

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