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Serotonin inhibits the delayed-rectifying K^+ (Kv) conductance in the epithelial cells of choroid plexus isolated from the rat

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Serotonin 5-HT_{2C} receptors are highly expressed in choroid plexus epithelial cells (Boess & Martin, 1994). Single channel experiments have shown that serotonin reduces the open probability of an 18 pS K^+ channel in mouse choroid plexus (Hung *et al.* 1993). In the present study we have examined the effects of serotonin on the delayed-rectifying K^+ (Kv) conductance expressed in rat choroid plexus cells (Kotera & Brown, 1994).

The choroid plexus was isolated from the fourth ventricle of rats killed humanely by overdose of halothane inhalation. K^+ channel activity was measured by conventional whole-cell methods using a K^+-rich pipette solution. Kv currents were measured at membrane potentials between −60 and +60 mV. In control conditions the maximum current density observed at +60 mV was 16.4 ± 2.0 pA pF^{-1} (mean ± S.E.M.; $n = 8$), immediately after attaining the whole-cell recording. Channel 'run-down' caused the current to decrease slightly to 15.0 ± 1.8 pA pF^{-1} over 8 min (91 ± 3 % of the maximum). By contrast, superfusion of the cells for 8 min, with a bath solution containing 1 μM serotonin caused current to decrease to 48 ± 5 % of the maximum ($n = 4$). The current density after 8 min (8.2 ± 1.1 pA pF^{-1} was significantly less than that measured in the control experiments ($P > 0.01$ by Student's t test for unpaired data).

Serotonin acts via 5-HT_{2C} receptors to activate phospholipase C causing an increase in intracellular [Ca^{2+}] and activation of protein kinase C (PKC). Previous studies have shown that the activity of the Kv channels in choroid plexus is not affected by changes in intracellular [Ca^{2+}] (Kotera & Brown, 1994). The effects of activating PKC on the Kv conductance were therefore examined. Choroid plexus cells were pretreated for 10–20 min with: 1 μM serotonin, 500 nM phorbol 12-myristate 13-acetate (a phorbol ester which activates PKC) or 30 μM 1,2-dioctanoyl-sn-glycerol (a membrane permeable analogue of diacylglycerol). The maximum current at +60 mV was significantly reduced compared to control by serotonin and by both of the PKC activators ($P < 0.01$ by ANOVA).

In conclusion, serotonin inhibits the Kv channels in choroid plexus cells, possibly by acting on 5-HT_{2C} receptors to activate PKC.

Boess FG & Martin IL (1994). *Neuropharmacology* **33**, 275–317.

Hung BC *et al.* (1993). *Brain Res* **617**, 285–295.

Kotera T & Brown PD (1994). *Pflugers Archiv* **237**, 317–324.

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All procedures accord with current UK legislation.

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Adenosine undergoes rapid metabolic degradation after the intracerebral injection into the rat brain

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The study was aimed to elucidate the metabolic fate of [^{14}C]adenosine after the injection into the brain and was performed on six Wistar rats (250 g).

The rats were anaesthetised (thiopenthone-Na, 40mg kg^{-1} I.P.), placed in a stereotaxic frame, a hole drilled in the skull, and the tip of a needle lowered into the left hemisphere to the point known as Par2 (Kakee *et al.* 1996). That needle was connected to micro syringe (Hamilton) and 0.5ml of artificial interstitial fluid containing 0.2 mCi of [^{14}C]adenosine was injected into the brain slowly over 2 s and then 0.5 ml of blood collected from the left jugular vein carefully between the 3rd and 5th minute after the intracerebral injection. These samples were centrifuged, plasma samples were boiled for 5 min and centrifuged again to precipitated proteins, and 20 ml of supernatant of each sample was used for liquid chromatography (HPLC) separation of purines, which was followed by simultaneous scintillation counting of radioactivity. The result revealed that (mean ± S.E.M.) 80.43 ± 11.23% and 7.11 ± 3.61% of total radioactivity in plasma appeared within peaks of hypoxanthine and adenine ($n = 3$), respectively, the two nucleobases that could be considered as the metabolic degradation products of adenosine. Only the background radioactivity could be detected within the adenosine spot. When 0.5 mM erythro-9-(2-hydroxy-3-nonyl)-adenosine, a potent inhibitor of adenosine deaminase, was injected into the brain simultaneously with [^{14}C]adenosine, 38.45±6.93% of total radioactivity in the plasma appeared within the adenosine peak ($n = 3$). Therefore, it seems that the blood–brain barrier represents a powerful enzymatic barrier for adenosine, and that the adenosine deaminase plays a central role in that function.

Kakee A *et al.* (1996). *J Pharmacol Exp Ther* **277**, 1550–1559.

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The use of cerebral NIRS monitoring during hypoperfusion events in cardiopulmonary bypass

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Cardiopulmonary bypass is frequently carried out under mild hypothermia since cooling is used to reduce metabolic rate and hence can offer protection from cerebral and myocardial ischaemia. However, there is increasing concern that rewarming following hypothermia may adversely affect the neurological outcome (Grigore *et al.* 2002). We have studied the cerebral effects of hypoperfusion events during cardiopulmonary bypass under mild hypothermia, and following rewarming using near infrared spectroscopy (NIRS). NIRS has been used to assess cerebral oxygen delivery, utilisation and perfusion.

We studied 10 patients who underwent coronary artery bypass grafting by the same surgeon. Ethical approval was granted and patient consent obtained. A standardised anaesthetic technique was used. Total body oxygen consumption was measured by continuous arteriovenous oximetry (Crerar-Gilbert *et al.* 2001). Cerebral oxygenation was measured simultaneously using a CRITIKON (Johnson & Johnson Medical) near infrared instrument (Thorniley *et al.* 1997). Statistical analysis was carried out using the independent samples t test (SPSS 10.0). Data are given as means ± S.D.

A total of 51 hypoperfusion episodes were recorded during hypothermia (32°C) and 20 episodes following rewarming to 37.5°C. The basal oxygen consumption at 32°C was 38.3 ± 17.0 ml min^{-1} m^{-2} increasing significantly to 71.5 ± 23.0 ml min^{-1} m^{-2} (mean ± S.D.) at 37.5°C ($P < 0.002$). Episodes of hypoperfusion resulted in a significant reduction in total body oxygen consumption, associated with significant reductions in the concentration of cerebral oxyhaemoglobin and total

haemoglobin ($P < 0.05$, 95% CI). Figure 1 shows a typical example of desaturation of oxyhaemoglobin during a hypoperfusion event. Oxyhaemoglobin concentrations decreased by -30.8 ± 17.6 μmol cm^{-1} per hypoperfusion episode at 32°C and -28.43 ± 16.4 μmol cm^{-1} at 37.5°C (mean ± S.D.).

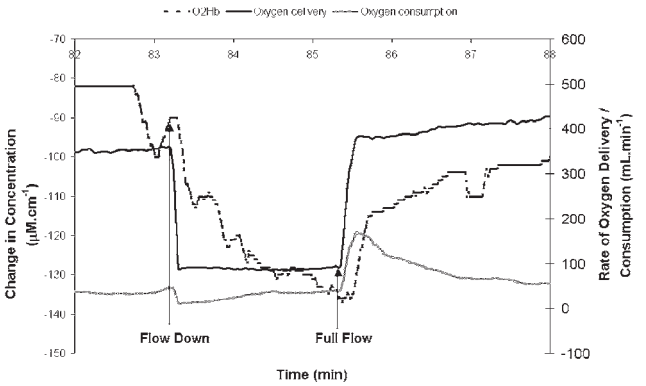

Figure 1. An example of desaturation of oxyhaemoglobin during a hypoperfusion event.

We believe that that these results albeit preliminary indicate that NIRS has an important role in cerebral monitoring during hypoperfusion.

Crerar-Gilbert A *et al.* (2001). *Br J Anaesth* **87**, 660–661.

Grigore AM *et al.* (2002). *Anaesth Analg* **94**, 4–10.

Thorniley MS *et al.* (1997). *Philos Trans R Soc Lond B Biol Sci* **352**, 685–696.

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