The effect of cyclosporine on taurine transport in human cord blood cells

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Cyclosporine has been used in long-term organ transplant treatment and there are reports of an increased occurrence of intrauterine growth retardation (IUGR) in pregnancies in such patients (Pickrell *et al.* 1988; Cockburn & Krupp, 1989). Indirect evidence suggests that this might result from an inhibitory effect of cyclosporine on taurine uptake by the placenta (Ramamoorthy *et al.* 1992). Here we investigated taurine transport by peripheral blood mononuclear cells (PBMs) enriched from cord blood (CB), an alternative and easily obtainable fetal tissue to placental tissue. We determined whether cyclosporine inhibited taurine uptake by these cells.

CB was obtained from placentas from normal term pregnancies in accordance with local ethical approval. PBMs were enriched by dilution with Hanks' biological salt solution (HBSS, containing (mM): NaCl 137, KCl 5.4, KH_2PO_4 0.4, Na_2HPO_4 0.34, $NaHCO_3$ 4.2 and glucose 5.6) and centrifuged at 400 *g* for 30 min (modified from Wan *et al.* 1999). [^{3}H] taurine uptake was measured with or without 10 mM β-alanine at 37 °C with or without pre-incubation with cyclosporine (5 μM), 37°C for 10 min using methods similar to those previously described (Ayuk *et al.* 2000). Data are expressed as means ± S.E.M.; n = number of placentas from which CB samples were taken.

Uptake of [^{3}H] taurine by CB PBMs was linear over 15 min (5.49 ± 0.92 fmol $(10^6$ cells$)^{-1}$ min^{-1}, $n = 6$), inhibitable by β-alanine (0.22 ± 0.09 fmol $(10^6$ cells$)^{-1}$ min^{-1}, $n = 6$ $P < 0.05$, Student's paired t test). Pre-incubation with cyclosporine (5 μM) inhibited [^{3}H] taurine uptake by 29.3 ± 5.3 % ($n = 8$, $P < 0.05$, Student's paired t test). There was no effect on the uptake of [^{3}H] taurine by methanol, the vehicle used to dissolve cyclosporine.

In conclusion, the effect of β-alanine on taurine uptake into CB PBMs suggests that this was mediated by system β, although further characterisation is required. The inhibitory effect of cyclosporine on taurine transport in CBCs was comparable to that seen in choriocarcinoma cells (Ramamoorthy *et al.* 1992). Thus the increased incidence of IUGR previously reported in mothers being treated with cyclosporine A might be due partially to effects on taurine uptake into fetal and placental tissues.

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The effect of 1-chloro-2,4-dinitrobenzene on K^+ transport in human red blood cells

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1-Chloro-2,4-dinitrobenzene (CDNB) lowers [reduced glutathione] and increases passive K^+ transport in human red blood cells (RBCs) (Shartava *et al.* 2000; Muzyamba *et al.* 2001). The major passive K^+ transport pathways are the K^+–Cl^- cotransporter (KCC) and the Ca^{2+}-activated K^+ channel (Gardos channel, I_K), but there is some discrepancy about which system represents the main target of CDNB.

Normal human RBCs (HbA cells) and RBCs from sickle cell patients (HbS cells), taken with ethical permission from consenting volunteers, were pretreated for 60 min at 10 % haematocrit (Hct) with or without CDBN (1 mM). They were then equilibrated in air or N_2 prior to measurement of transporter activity at 4 % Hct using $^{86}Rb^+$ as a K^+ congener, in the presence of ouabain (0.1 mM) and bumetanide (1 μM) to obviate transport via Na^+/K^+ pump and Na^+–K^+–Cl^- cotransporter. KCC activity was determined as the Cl^--dependent component of K^+ influx (Cl^- replaced with NO_3^-); I_K as the clotrimazole (5 μM)-sensitive component. $^{45}Ca^{2+}$ was used to measure maximal activity of the plasma membrane Ca^{2+} pump (PCMA) following the methodology of Tiffert *et al.* (1993). Data are given as means ± S.E.M. ($n = 3$), fluxes as mmol K^+ or Ca^{2+} (l cells h)$^{-1}$.

In oxygenated HbA cells (K^+ 5 mM, Ca^{2+} 2.5 mM), KCC activity increased from 0.24 ± 0.04 in controls to 13.22 ± 2.67 with CDNB, and I_K activity from 0.20 ± 0.14 to 5.82 ± 1.54; in N_2, activities in CDNB-treated cells were 3.12 ± 1.65 for KCC and 1.11 ± 0.56 for I_K. Similar effects were observed in oxygenated HbS cells, but there was no inhibition on deoxygenation. In oxygenated CDNB-treated cells, removal of extracellular Ca^{2+} (Ca^{2+}-free saline plus 50 μM EGTA) inhibited the activity of KCC by 93 ± 1 %, and there was no observable I_K activity. The protein phosphatase inhibitor, calyculin A (100 nM), inhibited KCC activity by 93 ± 5 % when added prior to CDNB, but had no effect when added afterwards. I_K activity was unaffected by calyculin A. [ATP] and Na^+/K^+ pump activity were unaffected by CDNB. Finally, PMCA was reduced by 33 ± 1 % on exposure to CDNB.

The results demonstrate that CDNB stimulates both KCC and I_K. Stimulation is dependent on extracellular Ca^{2+}. Inhibition of PCMA may contribute to activation of I_K. Stimulation of KCC is consistent with inhibition of a regulatory protein kinase, but there is no evidence of depletion of total or membrane-bound pools of ATP.

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The effect of nitrite on passive K^+ transport in human red blood cells

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Red blood cells (RBCs) from sickle cell patients, which are exposed to a greater oxidative challenge than normal, have elevated K^+ transport. This characteristic is responsible for their dehydration. As part of a study to investigate the mechanisms responsible for this transport phenotype, we have examined the effects of oxidants on passive K^+ transport in RBCs (Muzyamba *et al.* 2000, 2001). Stimulation of the K^+–Cl^- cotransporter (KCC) correlated with both reduction of reduced glutathione (GSH) and accumulation of methaemoglobin (metHb), not GSH alone, whilst loss of O_2 dependence of KCC was independent of GSH but correlated with accumulation of metHb. Here we report the effects of nitrite (NO_2^-), which stimulates KCC in RBCs from sheep and horse (Adragna & Lauf, 1998; Muzyamba *et al.* 2000), on human RBCs.

Normal human RBCs, taken with ethical permission from consenting volunteers, were pretreated for 60 min at 10 % haematocrit (Hct) with or without NO_2^- (1, 3 or 5 mM). Cells were then equilibrated in air or N_2 after which transporter activity (80 mM K^+, 2.5 mM Ca^{2+}) was measured at 4 % Hct using $^{86}Rb^+$ as a K^+ congener, in the presence of ouabain (0.1 mM) and bumetanide (1 μM) to obviate transport through Na^+/K^+ pump and Na^+–K^+–Cl^- cotransporter. KCC activity was determined as the Cl^- -dependent component of K^+ influx (Cl^- replaced with nitrate). We also measured activity of the Ca^{2+}-activated K^+ channel (Gardos channel or I_K) as the clotrimazole (5 μM)-sensitive component.

Increasing [NO_2^-] produced progressive stimulation of KCC (3.5-fold at 1 mM; 6.5-fold at 5 mM) and loss of O_2 dependence (deoxygenation inhibited KCC in control cells by 90 % but only about 50 % in NO_2^--treated cells) (Table 1; $P < 0.05$, Student's paired t test). As for other oxidants, treatment with NO_2^- caused accumulation of metHb (> 60 % at 5 mM) and depletion of GSH. I_K, however, was not activated in either control cells or following treatment with NO_2^-.

Table 1. The effect of nitrite on K^+–Cl^- cotransport in human red blood cells (RBCs)

	Oxygenated RBCs	Deoxygenated RBCs
Control	1.27 ± 0.10	0.13 ± 0.13
1 mM nitrite	4.46 ± 0.65	2.14 ± 0.55
5 mM nitrite	8.23 ± 2.93	3.86 ± 0.31

Activity of the K^+–Cl^- cotransporter was determined as Cl^--dependent K^+ influx (Cl^- replaced by NO_3^-), using $^{86}Rb^+$ as a K^+ congener, in the presence of ouabain (0.1 mM) and bumetanide (1 μM). Influxes, in mmol (l cells h)$^{-1}$, are given as means ± S.D., $n = 3$.

In conclusion, NO_2^-, like other oxidants, has profound effects on membrane transport in human RBCs altering the response to O_2 tension. Elucidating the target of oxidants will be invaluable for understanding the transport abnormalities observed in sickle cells.

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