Placenta (1992), 13, 231–240

Effect of Maternal Hypokalaemia on Unidirectional Maternofetal and Net Potassium Fluxes Across the Placenta of the Anaesthetized Rat

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Paper accepted 30.10.1991

SUMMARY

Potassium (K^+) fluxes across the placenta of rats, at 21 days gestation, fed a low K^+ diet or a control diet were studied. The rats on the low K^+ diet had a significantly ($P < 0.001$) lower arterial plasma K^+ concentration compared to those on the control diet (1.95 ± 0.12 and 2.93 ± 0.06 mmol/l respectively; mean ± s.e., n = 17). Fetal umbilical arterial plasma K^+ concentration was unaltered in maternal hypokalaemia and was significantly ($P<0.001$) higher than that of maternal plasma (4.58 ± 0.15 and 4.66 ± 0.12 mmol/l in low K^+ and control groups respectively). Net K^+ flux across the placentas (as measured by fetal accretion between days 20 and 21 of gestation) of hypokalaemic mothers (0.106 ± 0.02 μmol/min/g placenta, n = 6) was not different to that in controls (0.104 ± 0.01 μmol/min/g placenta, n = 8). Unidirectional maternofetal flux (J_{mf}) across the placentas, measured as the accumulation of ^{42}K in the fetuses after injection of the radioisotope into maternal blood, was also not significantly different between the hypokalaemic and control mothers (0.5 ± 0.08 μmol/min/g placenta, n = 8 versus 0.63 ± 0.06 μmol/min/g placenta, n = 7, respectively). However, measurement of J_{mf} by perfusion of the placentas through their fetal circulations yielded a higher value than the accumulation method and in this analysis was significantly ($P < 0.02$) lower in the low K^+ than in the control group (0.75 ± 0.10 μmol/min/g placenta, n = 11, and 1.27 ± 0.14 μmol/min/g placenta, n = 9, respectively). These results show that net placental K^+ fluxes are unaltered during maternal hypokalaemia but suggest that unidirectional maternofetal fluxes may be reduced.

0143–4004/92/030231 + 10 $05.00/0

INTRODUCTION

There have been a number of studies which suggest that fetal plasma K^+ concentrations remain unaltered or even increase during severe maternal hypokalaemia. Serrano, Talbert and Welt (1964) found in the dog that under control conditions the fetal plasma K^+ concentration was higher than maternal (5.1 mM versus 4.1 mM) and that when dietary K^+ restriction reduced maternal concentrations to 2.7 mM the fetal plasma concentration actually increased to 5.9 mM. Dancis and Springer (1970) made a similar observation in the rat: when maternal venous plasma K^+ concentration was lowered from 4.6 ± 0.25 mM to 2.3 ± 0.18 mM by means of feeding a low K^+ diet, fetal plasma (axilliary vessel) K^+ concentration was not significantly altered (5.5 ± 0.15 mM in the control group, 6.0 ± 0.27 mM in the low K^+ group).

One of the problems in such measurements is that hypoxia and maternal anaesthesia can significantly increase movement of K^+ out of cells and thereby artefactually raise fetal levels (Battaglia et al, 1958). Bearing this in mind Fantel (1975) took particular care in taking fetal samples from umbilical vessels using a micro-capillary method and found that, nevertheless, fetal K^+ concentration rose with increasing time from laparotomy. This emphasizes the critical importance of methodological detail in studies of plasma K^+ concentrations. When Fantel extrapolated his data to zero time to give an indication of the 'true' fetal plasma K^+ concentration prior to laparotomy it was found to be lower than in maternal plasma. However, using the same methods, Fantel (1978) found that during maternal hypokalaemia fetal plasma K^+ concentration became significantly higher than maternal in agreement with the studies of Serrano, Talbert and Welt (1964) and Dancis and Springer (1970).

There would seem to be two possible explanations, not mutually exclusive, for this maintenance or even increase in fetal plasma K^+ concentration during maternal hypokalaemia. Firstly, it might simply result from a redistribution of fetal K^+ from other tissues. However, the evidence is that total fetal K^+ concentration does not change, or decreases only slightly, when compared to the large decrease in maternal muscle K^+ concentration during maternal hypokalaemia (Stewart and Welt, 1961; Serrano, Talbert and Welt, 1964; Dancis and Springer, 1970). Secondly, it is possible that there are alterations in placental K^+ fluxes so as to protect the fetus from maternal hypokalaemia to some extent. It is this second possibility which we have addressed in the investigation reported here with respect to net and unidirectional maternofetal fluxes.

In our study, rats were fed control or K^+ deficient diets similar to those used by Dancis and Springer (1970) and maternal and fetal plasma K^+ concentrations were measured in samples obtained by a method similar to that of Fantel (1975) so as to reduce artefacts as far as possible. Net maternofetal K^+ flux was calculated for the two dietary groups from their increase in fetal plasma K^+ concentration with gestation. Unidirectional maternofetal clearances and fluxes were measured both by measuring accumulation of ^{42}K by the intact fetus after maternal injection (cf Flexner and Pohl, 1941) and by perfusion of the fetal circulation of the placenta in situ (Štulc and Štulcová, 1986; Robinson et al, 1988).

METHODS

Dietary manipulation

Female Sprague Dawley rats were maintained on a normal rat pellet diet (CRM rat diet; Special Diet Services, Manea, Cambridge, UK) prior to mating. The diet was then changed

for the remainder of the experiments to either a reconstituted rat diet which was low in K^+ content (low K^+ diet) or to the same diet to which 50 mmol/Kg of potassium sulphate had been added (control diet). Both diets were prepared commercially by BP Nutrition (Special Diet Services, Witham, Essex, UK) and were identical in every other way apart from the added potassium sulphate; analysis showed that the low K^+ diet contained 0.02 g K^+/100 g diet whereas the control diet contained 0.92 g/100 g.

Where experiments were performed comparing the two dietary groups, an approximately equal number of rats from each group were studied each week to allow for any seasonal or systematic variation. Experiments were performed on day 21 of gestation, day 1 being the day on which the vaginal plug was found (term is 23 days), for measurement of maternofetal flux and maternal and fetal plasma K^+ determinations. In the experiments in which net flux was determined as fetal K^+ accretion the fetuses were taken at the same time on days 20 and 21 of gestation.

Preparation of animals for experiments

The pregnant rats were initially anaesthetized with ether. When the animal lost consciousness, anaesthesia was continued with intraperitoneal injection of 110 mg/kg sodium thiobutabarbital (Inactin, BYK Gulden, Hamburg, Germany). The rat was transferred to the experimental bench and 1 ml of lignocaine hydrochloride (1 per cent w/v) was injected superficially into the neck. The trachea was then exposed and cannulated using a 5 cm long 1.5 mm bore pvc tube to ease breathing. A jugular vein was cannulated for the infusion of radioisotopes using a 30 cm long, 2 French gauge cannula (Portex, Hythe, UK) and a carotid artery was cannulated using a 3 French gauge Portex cannula to allow blood sampling and blood pressure monitoring via a pressure transducer (Gould) connected to a chart recorder. The cannulae were kept patent throughout the experiment by occasional flushing with 10 U/ml heparin in 0.9 per cent saline. The animals were kept warm using a dissection lamp. Maternal body temperature was not measured in experiments in which only fetal blood samples were taken, but in other experiments maternal rectal temperature was monitored and kept to within one degree of 37°C.

Measurement of maternal and fetal plasma K^+ concentrations

Maternal blood samples were obtained from the maternal carotid artery using a syringe attached to the cannula; approximately 1 ml of blood was removed before taking maternal samples to avoid contamination with the flush solution (10 U/ml heparin in 0.9 per cent saline). A laparotomy was then performed and the time noted. A small cut was made in the uterus and after isolating a single fetus by gently pulling it out of the uterus, a fetal blood sample (about 0.5 ml) was obtained from a pulsating umbilical artery using a cut needle attached to a heparinized capillary tube. The other end of the capillary tube was attached to polyethylene tubing allowing the operator to slowly aspirate the blood sample into the capillary tube. The needle was then removed from the capillary tube and the blood sample was fed directly into the KNA2 ion selective Na^+ and K^+ analyser (Radiometer, Copenhagen); the electrodes in this instrument are highly selective and there is no interference in K^+ measurements by Na^+ or by H^+ at physiological pH. As many fetuses as possible were sampled over a 30–60 min period. Samples were discarded if they were difficult to obtain, insufficient for reliable results from the KNA2 analyser or were obtained from non-pulsating arteries. Maternal samples were also obtained from maternal uterine arteries of the same calibre as the fetal umbilical artery at the end of the experiment using the same technique.

Accretion studies

Rats fed the control diet and low K^+ diet were studied on days 20 and 21 of gestation. The rats were weighed, anaesthetized and trachea, jugular and carotid vessels cannulated as above. A maternal blood sample was taken from the carotid artery cannula for plasma K^+ concentration measurement. A laparotomy was performed and six fetuses were removed at random and were separated from their membranes and placentas. The total volume of amniotic fluid was determined by carefully weighing the intact feto-placental unit together with intact sacs and then removing all amniotic fluid and reweighing. A sample of amniotic fluid was collected using a 21 gauge needle and immediately analysed with the KNA2 analyser. Mean values of fetal weight, placental weight, membrane weight and amniotic fluid volume were determined for each rat.

Three fetuses out of the six removed from each rat were weighed and placed in individual crucibles and ashed in an oven at 800°C for 5 to 6 h. After cooling, the contents of each crucible were dissolved in 3 ml of 3 M nitric acid and further diluted in distilled water to ten times the original wet weight of fetus. A sample of this was analysed for K^+ concentration using the Perkin-Elmer Atomic Absorption Spectrophotometer. The results were expressed as μmol/g wet weight of fetus. Three placentas and approximately 1 g of maternal quadriceps muscle from each animal were also analysed in a similar manner. Three to six fetal amniotic and allantoic membranes from each rat were pooled and ashed in one crucible and analysed in a similar way. Mean K^+ concentrations in whole fetuses, maternal muscle, whole placentas and fetal membranes were thus determined.

For each animal the mean fetal K^+ content was calculated and the net accretion rate per fetus (J_{net}) was calculated for the two dietary groups by dividing the difference between the K^+ content per fetus for each animal at 20 days gestation and the mean K^+ content at 21 days by the time difference (24 h).

Clearance and flux measurements using ^{42}K

Rats at 21 days gestation were anaesthetized as above and the trachea, carotid artery, and jugular vein cannulated.

^{42}K was prepared from KCO_3 in the University of Manchester reactor. Specific radioactivity immediately after preparation was approximately 1 μCi/μmol and a stock solution was prepared by diluting 200 μCi in 10 ml of 0.9 per cent saline. A steady level of ^{42}K in maternal plasma was established and maintained by an intravenous injection of a priming dose of ^{42}K (approximately 10 μCi) followed by a continuous infusion at a decreasing rate (as determined in preliminary experiments). Although the specific activity of the ^{42}K was initially high, the short half-life of the radioisotope (12.5 h) meant that this was considerably lower in experiments carried out 24–48 h after its preparation with consequent small but significant effects on maternal plasma K^+ concentrations (see Results).

Transplacental flux and clearance of ^{42}K was determined by measurement of radioactivity in the umbilical venous effluent of fetal side perfused placentas as well as by measurement of total radioactivity accumulated in fetuses of intact (unperfused) placentas as detailed below. The fetal accumulation measurements were always performed in the same animals as measurements across perfused placentas by taking fetuses from the non-perfused horn at the end of perfusion. In some experiments only perfusion measurements were made.

Measurement of maternofetal clearance ($^{perf}K_{mf}$) *and flux* ($^{perf}J_{mf}$) *during umbilical perfusion.* A method modified from that of Štulc and Štulcová (1986) as described by Robinson et al (1988) was used to perfuse the rat placenta through its fetal circulation. The rat was

immobilized on its back and kept warm using a dissection lamp or in some experiments by a water heater (kept at 37°C by warm water circulation) under the rat. After laparotomy one fetus was delivered through a small incision in the uterus in such a way that the uterus retracted and enveloped the placenta, preventing placental separation. Care was taken not to touch the umbilical cord unnecessarily as manipulation was often noted to lead to rapid constriction of its vessels. The umbilical cord was gently supported over a platform constructed from plastic building bricks ('Lego').

The umbilical artery (there is only one in the rat) was cannulated using a steel needle (0.5 mm O.D.) attached to Silastic tubing (0.63 mm O.D., 0.3 mm I.D., Dow Corning Corporation, Michigan, USA) and the umbilical vein was then cannulated using a 24 gauge Jelco catheter (Critikon, Tampa, USA). The cannulae in both vessels were tied together and incorporated in a suture used to occlude the vitelline vessels. The fetal circulation was perfused with a modified Krebs Ringer solution containing (mM) Na^+ 143, K^+ 4.7, Cl^- 126, HCO_3^- 25, Ca^{2+} 1.25, PO_4^{2-} 1.0, SO_4^{2-} 1.1, Mg^{2+} 1.1, antipyrine (Sigma Chemical Co plc) (as a flow limited marker) 0.5 g/l, glucose 2 g/l, dextran (40 000 MW) 35 g/l. This solution was gassed with 95 per cent O_2/5 per cent CO_2, to pH 7.4 warmed at 37°C and perfused via the umbilical artery at a rate of 0.5 ml/min. Experiments where blood pressure fell below 65 mmHg or where perfusate recovery was less than 95 per cent of inflow rate were not continued with.

Administration of ^{42}K was started about 5 min after the onset of the perfusion. Maternal blood samples (0.5 ml) were taken at 10 min intervals, 0.1 ml aliquots used for measurement of K^+ concentrations (KNA2) and the remainder centrifuged immediately to obtain plasma for measurement of radioactivity. Umbilical venous effluent was collected from the umbilical vein cannula at 5-min intervals after a single passage through the placenta. A single bolus of ^{51}Cr-EDTA (0.5 ml, 20 μCi; used to determine paracellular diffusional permeability) was also injected into the maternal circulation at 25 min after the start of perfusion. After 60 min the perfusion was ended and the perfused placenta removed, trimmed and weighed.

$^{perf}K_{mf}$ was calculated for each 5 min collection period:

$$^{perf}K_{mf} = \frac{[v] \times Q}{[A] \times Wt} \mu l/min/g \text{ placenta} \quad (1)$$

where $[v]$ = Radioactivity in perfusate effluent (ct/min/ml).
$[A]$ = Radioactivity in maternal arterial plasma (ct/min/ml) at the midpoint of the collection period (graphically interpolated).
Q = Perfusate flow rate (μl/min) determined by weighing the timed effluent collections.
Wt = Placental wet weight (g).

$^{perf}J_{mf}$ for K^+ was calculated by multiplying $^{perf}K_{mf}$ by the mean maternal plasma K^+ concentration during the study. In preliminary experiments it was found that $^{perf}K_{mf}$ for K^+ was relatively constant from 30 min onwards and so steady-state values were calculated as the mean of 30, 35 and 40 min samples. It has previously been shown that $^{perf}K_{mf}$ for ^{51}Cr-EDTA rapidly (<4 min) achieves a steady state (Robinson et al, 1988) and the mean of the same samples was also calculated for this tracer. Fetomaternal clearance ($^{perf}K_{fm}$) of antipyrine was calculated for similar 5 min collection periods as:

$$^{perf}K_{fm} = \frac{([a] - [v]) \times Q}{[a] \times Wt} \mu l/min/g \text{ placenta} \quad (2)$$

where $[a]$ = the antipyrine concentration in inflowing perfusate.
$[v]$ = the antipyrine concentration in effluent perfusate.

It was assumed that at steady state it would be identical with the maternofetal clearance.

Measurement of maternofetal clearance ($^{intact}K_{mf}$) *and flux* ($^{intact}J_{mf}$) *by fetal accumulation of radioisotope.* A method based on that originally devised by Flexner and Pohl (1941) was used to determine unidirectional maternofetal clearance ($^{intact}K_{mf}$) and flux ($^{intact}J_{mf}$) across intact placentas. To do this, a radioactive tracer was injected into the maternal circulation and, after a known period, a fetus was removed and the amount of radioactivity in the carcass measured. Preliminary experiments were performed to measure fetal plasma ^{42}K concentration in pooled fetal blood at the end of 60 min. This was found to be between 12 and 20 per cent of maternal plasma concentration (mean ± s.e.; 15 ± 3 per cent; $n = 4$). Also in preliminary experiments amniotic fluid was obtained and its ^{42}K content found to be less than 1 per cent of the total fetal content. Amniotic fluid radioactivity was therefore not measured in the remainder of the experiments.

Studies using this approach were made simultaneously with perfusion experiments. At the end of the 60 min the entire uterine horn (opposite to the one containing the perfused placenta) was immediately removed from the rat. Three fetuses were selected at random from this horn, removed, blotted gently and weighed. The fetal and maternal plasma radioactivity was measured as described below.

$^{intact}K_{mf}$ was determined as:

$$^{intact}K_{mf} = \frac{F}{[A] \times Wt \times 60} \mu l/min/g \text{ placenta} \quad (3)$$

where F = the mean total radioactivity content of the fetus taken at 60 min from the time of ^{42}K injection, (ct/min/fetus),
$[A]$ = the mean maternal plasma radioactivity (mean of six samples taken at 10, 20, 30, 40, 50 and 60 min; ct/min/μl).

$^{intact}J_{mf}$ was calculated by multiplying the K_{mf} value by the mean maternal plasma K^+ concentration during the 60-min period.

Analytical techniques

For estimation of antipyrine concentration, perchloric acid (0.1 ml, 3 M) was added to 0.9 ml of perfusate to precipitate any proteins. The mixture was then centrifuged for 2 min and the supernatant removed. Chloroform (3 ml) was added to 0.5 ml of this supernatant and the resulting mixture was vigorously shaken. Absorbance in aliquots of the chloroform phase was measured at 340 nm and concentration determined from a standard curve. Gamma radioactivity was measured in 1 ml of perfusate and in 0.1 ml of maternal plasma made up to a volume of 1 ml with distilled water. Maternal plasma samples were counted in duplicate. Samples were also recounted after ^{42}K decay to determine ^{51}Cr-EDTA activity. Fetuses were cut up into several pieces and put into one or more counting tubes so as to make an approximate volume of 2.5 ml per tube. All samples were counted for 2 min (Packard Autogamma 800) and corrected for background and ^{42}K decay during the counting period.

Statistics

Data is shown in all cases as mean ± the standard error of the mean (s.e.). Statistical comparisons were made using the Mann–Whitney U-test for unpaired data and the

Table 1. Effects of a low K^+ diet on various measurements in the rat at 21 days gestation (mean ± s.e.)

	Control diet (n)		Low K^+ diet	
Rat weight (g)	360 ± 10	(17)	317 ± 13[c]	(17)
Fetal weight (g)	3.37 ± 0.06	(17)	3.01 ± 0.12[b]	(17)
Placental weight (g)	0.42 ± 0.01	(17)	0.41 ± 0.01	(17)
Fetal K^+ concentration (mmol/kg)	46.0 ± 1.3	(17)	47.0 ± 1.5	(17)
Total fetal K^+ content (μmoles)	162.5 ± 3.8	(17)	148.0 ± 5.6[a]	(17)
Placental K^+ concentration (mmol/kg)	39.0 ± 2.2	(16)	34.3 ± 1.4	(15)
Maternal muscle K^+ concentration (mmol/kg)	84.8 ± 1.8	(17)	71.9 ± 2.7[d]	(17)
Maternal plasma K^+ concentration (mmol/l)	2.93 ± 0.06	(17)	1.95 ± 0.12[d]	(16)
Fetal plasma K^+ concentration (mmol/l)	4.66 ± 0.12[e]	(17)	4.58 ± 0.15[e]	(16)

[a] $P = 0.52$; [b] $P < 0.05$; [c] $P < 0.01$; [d] $P < 0.001$ versus control diet (Mann–Whitney U-test).
[e] $P < 0.001$ versus maternal plasma K^+ concentration (paired t-test).
All tissue weights are net weights.

Student's t-test for paired data. Where more than one fetus or placenta was analysed in each animal results were expressed as mean value per rat (i.e. n = number of mothers).

RESULTS

Effects of a low K^+ diet on maternal and fetal plasma K^+ concentrations

Table 1 shows the effects of dietary K^+ content on a variety of measurements on the pregnant rats in this study. As reported by Dancis and Springer (1970) the animals on low K^+ generally looked less healthy and both maternal and fetal weights were lower ($P < 0.02$) than controls. Maternal muscle K^+ concentration was lower in the K^+-deficient animals as compared to control but fetal K^+ concentration (mmol/Kg fetal tissue) was not significantly reduced. There was a barely significant ($P = 0.052$) decrease in total fetal K^+ content arising from the fetuses being smaller. Neither placental weight nor placental K^+ concentration was affected by the low K^+ diet.

Maternal plasma K^+ concentrations in samples taken from small uterine arteries were identical to samples taken at the same time from the maternal carotid cannula (data not shown) and these were significantly lower in rats fed the low K^+ diet as compared to those on the control diet (Table 1). We found no significant correlation between fetal plasma K^+ concentration and time after laparotomy for either dietary group (data not shown) therefore a mean value for each rat was obtained using all timed samples from the fetuses in that animal. As can be seen in Table 1 fetal plasma K^+ concentrations were unaltered by maternal hypokalaemia and were significantly higher than maternal concentrations in both dietary groups.

Net K^+ flux

The total fetal K^+ content (including all extraplacental membranes and amniotic fluid) in control and hypokalaemic rats at days 20 and 21 of gestation are shown in Table 2. It can be seen that J_{net} was not significantly different between the two groups.

Maternofetal clearances and fluxes across intact placentas

The data in Table 3 shows that neither ${}^{intact}K_{mf}$ nor ${}^{intact}J_{mf}$ for K^+ were significantly different between the control and low K^+ diet groups. The mean maternal plasma K^+

Table 2. Fetal K^+ content at 20 and 21 days gestation and calculated J_{net} for K^+ (mean ± s.e.)

	Control diet (*n*)	Low K^+ diet
Placental Wt		
20 d gestation (g)	0.40 ± 0.02 (8)	0.40 ± 0.04 (6)
21 d gestation (g)	0.42 ± 0.01 (17)	0.41 ± 0.01 (17)
Fetal K^+ content		
20 d gestation (μmoles)	98.7 ± 5.6 (8)	86.6 ± 8.4 (6)
21 d gestation (μmoles)	162.5 ± 3.8 (17)	148.1 ± 5.6 (17)
J_{net} (μmol/min/g placenta)	0.104 ± 0.01 (8)	0.106 ± 0.02 (6)

concentration was higher in these experiments (Table 3; see also Table 4) than in the first series (Table 1) mainly due to the use of ^{42}K after it had gone through two or three half lives, with consequent lower specific activity, on some occasions.

Maternofetal clearances and fluxes across perfused placentas

Comparison of Tables 3 and 4 shows that $^{perf}K_{mf}$ for K^+ was higher than $^{intact}K_{mf}$. $^{perf}K_{mf}$ in the control group was not significantly different from that for the low K^+ diet group. However, $^{perf}J_{mf}$ was significantly lower with the low K^+ diet.

DISCUSSION

In this study we have confirmed the presence of the fetomaternal plasma K^+ concentration difference in the rat reported by Dancis and Springer (1970). We have also confirmed the observation of Dancis and Springer (1970) that fetal plasma K^+ concentrations are maintained in maternal hypokalaemia. There still remains the speculative possibility that fetal K^+ levels were artefactually raised, perhaps due to excessive leakiness of fetal red cells. However, we believe this possibility has been minimized by our method of fetal blood sampling from a pulsating artery. This is supported by our failure to measure any increase in fetal K^+ concentration with time after laparotomy in contrast to the reports of Fantel (1975, 1978).

Whole fetal K^+ concentration was unaltered by maternal hypokalaemia and total fetal content was only slightly reduced (due to lower fetal weight) whereas maternal muscle K^+ concentration was markedly decreased. Placental weight, placental K^+ concentration and net K^+ flux to the fetus in late gestation were unchanged. It appears that the placenta itself protects the fetus from hypokalaemia although fetal growth is impaired.

Table 3. Unidirectional maternofetal clearance ($^{intact}K_{mf}$) and flux ($^{intact}J_{mf}$) of ^{42}K across intact rat placenta (mean ± s.e.)

	Control diet (n = 7)	Low K^+ diet (n = 8)
$^{intact}K_{mf}$ (μl/min/g placenta)	160 ± 16	235 ± 31
Maternal plasma K^+ concentration (mmol/l)	4.02 ± 0.16	2.13 ± 0.38[a]
$^{intact}J_{mf}$ (μmol/min/g placenta)	0.63 ± 0.06	0.50 ± 0.08

[a] $P < 0.001$ versus control diet (Mann–Whitney U-test).

Table 4. Unidirectional maternofetal clearances ($^{perf}K_{mf}$) for ^{42}K and $^{51}CrEDTA$, unidirectional fetomaternal clearance ($^{perf}K_{fm}$) for antipyrine and unidirectional maternofetal flux ($^{perf}J_{mf}$) for ^{42}K across perfused rat placenta (mean ± s.e.)

	Control diet ($n = 9$)	Low K^+ diet ($n = 11$)
$^{perf}K_{mf}$ ^{42}K (μl/min/g placenta)	317 ± 39	290 ± 21
Maternal plasma K^+ concentration	4.06 ± 0.13	2.54 ± 0.24[b]
$^{perf}J_{mf}$ ^{42}K (μmol/min/g placenta)	1.27 ± 0.14	0.75 ± 0.10[a]
$^{perf}K_{mf}$ $^{51}CrEDTA$ (μl/min/g placenta)	5.1 ± 0.5	6.5 ± 0.6
$^{perf}K_{fm}$ antipyrine (μl/min/g placenta)	527 ± 53	538 ± 64

[a] $P < 0.02$; [b] $P < 0.01$ versus control diet (Mann–Whitney U-test).

Net flux (J_{net}) of any solute across the placenta is the result of differences between the unidirectional maternofetal (J_{mf}) and fetomaternal (J_{mf}) fluxes (see Sibley and Boyd, 1988). In this study we measured J_{mf} for K^+ by two methods and obtained somewhat different results. $^{intact}J_{mf}$ was some 50 per cent of $^{perf}J_{mf}$ measured in the same rat (Tables 3 and 4). Both sets of measurements may have been affected by anaesthesia or by the surgical procedures employed and each has inherent potential errors which may explain at least partly the discrepancy between the two approaches. Thus measurements of $^{intact}J_{mf}$ are certainly underestimates because of the non-steady state conditions of ^{42}K transfer during the first 20–30 min. A rough estimate of the error can be made from the time course of K_{mf} in the perfused placenta by dividing the mean K_{mf} during the whole experiment by the apparent steady-state value (the mean of K_{mf} values from 30 min on). Estimated in this way, $^{intact}K_{mf}$ could be underestimated by a factor of 0.89 in the control group and 0.93 in the low K^+ group. In addition the calculation of maternofetal transfer of K^+ does not take into account the possible back flux of ^{42}K from fetus to mother. At the end of the 60-min period the fetal plasma radioactivity was, on average, 15 per cent of maternal plasma radioactivity. Assuming that the increase in fetal plasma radioactivity was approximately linear and that the movement of K^+ across the placenta is symmetrical (not quite true), the true value of $^{intact}K_{mf}$ should be about 8 per cent higher than the value calculated as described in Methods.

Conversely $^{perf}J_{mf}$ might be an overestimate because the permeability of the placenta to hydrophilic molecules may be increased by perfusion as shown by Hedley and Bradbury (1980) in the guinea-pig. However, no such effect has been found previously in the rat (Robinson et al, 1988). Furthermore the very low $^{perf}K_{mf}$ for ^{51}Cr-EDTA relative to the $^{perf}K_{mf}$ for ^{42}K in the present experiments indicates that any increase in placental permeability due to the perfusion would have only a negligible effect on K_{mf} for ^{42}K. We cannot exclude the possibility of an effect of perfusion on a transplacental route available to K^+ but not ^{51}Cr-EDTA. Neither $^{perf}K_{mf}$ for ^{51}Cr-EDTA nor $^{perf}K_{fm}$ for antipyrine were significantly different between the two dietary groups (Table 4) suggesting that there were no differences in passive permeability or maternal blood flow through the placenta respectively.

There was a significant reduction in $^{perf}J_{mf}$ for K^+ during maternal hypokalaemia which was not shown for $^{intact}J_{mf}$. However, there was a hint of a reduction in the latter during hypokalaemia, especially bearing in mind the steady-state considerations discussed above. If there really were a reduction in J_{mf} during hypokalaemia then, taken together with the preserved J_{net} for the ion in late gestation, there is the implication that there must be a larger decrease in J_{fm} for K^+ in the hypokalaemic pregnancies. This implication has not been directly addressed in the present study.

The mechanisms by which K^+ crosses the placenta are unclear. Any simple relationship

between maternofetal and transplacental potential difference is unlikely (McNaughton and Power, 1991) but as the maternofetal potential difference (p.d.) is fetus positive in the rat (Mellor, 1969) it seems unlikely that a p.d. will contribute to fetal K^+ acquisition. Evidence that K^+ transport is not purely passive in the rat is found in the observation that the ratio of ${}^{perf}K_{mf}$ for K^+ and for ^{51}Cr-EDTA (Table 4) is approximately 50 whereas that of their diffusion coefficients in water is only 3 (Hedley and Bradbury, 1980) suggesting a route for K^+ not accessible to ^{51}Cr-EDTA. Furthermore the higher fetal than maternal plasma K^+ concentrations and the net K^+ flux towards the fetus found in this study implies that active transport is involved. In the guinea-pig, Bailey et al (1979) and Hedley and Bradbury (1980) reached a similar conclusion.

A reduction in J_{mf} for K^+ in maternal hypokalaemia could result from an active transport system of low affinity for K^+ but the postulated decrease in J_{fm} cannot be explained in this way as there was no change in fetal plasma K^+ concentration. Perhaps there is more complex control of J_{fm} so as to preserve both J_{net} for K^+ and fetal plasma K^+ concentrations.

ACKNOWLEDGEMENTS

We are grateful to the staff of the University Animal Unit for their skilled help in this study and to J. D. Glazier for assistance and discussion. This work was supported by the North Western Regional Health Authority and a National Fund for Research into Crippling Diseases Endowment.

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