KIDNEY FUNCTION AND NET GLUCOSE PRODUCTION IN NORMAL AND ACIDOTIC SHEEP

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Abstract

Net glucose production and renal function were assessed simultaneously in normal sheep which were subsequently made acidotic by ammonium chloride administration. Arteriovenous differences in glucose concentration were measured, together with *p*-aminohippuric acid and inulin clearances in one kidney. In normal sheep there was a small net output of glucose by the kidney [20 μg min^{-1} (kg body weight)$^{-1}$] which was increased threefold during acidosis. The increase was associated with an elevated venous–arterial difference in glucose concentration and a fall in renal blood flow, while glomerular filtration rate did not change. The increase in the proportion of blood being filtered indicates that there is a greater perfusion of the active zone of the cortex, which is the major site of glucose production in the kidney. This suggests that changed intrarenal events are associated with the physiological response of the kidney to altered acid–base status. The contribution by the kidneys to the overall glucose requirements in sheep, even during acidosis, is unlikely to be greater than 6%.

I. INTRODUCTION

In mammals both the liver and kidney have the capacity to produce glucose from non-carbohydrate precursors. Krebs (1963) examined renal gluconeogenesis under *in vitro* conditions and concluded that the kidneys could make a significant contribution to glucose production.

Glucose production was increased in kidney cortex slices from rats and dogs which had previously been made acidotic by ammonium chloride ingestion (Goodman *et al.* 1966; Goorno *et al.* 1967; Kamm *et al.* 1967). However, *in vivo* studies using anaesthetized dogs showed no net output of glucose by the kidney in control experiments, whereas a significant output was measured during acidosis (Steiner *et al.* 1968). The finding was not confirmed by Roxe *et al.* (1970) using ^{14}C-labelled glucose to monitor glucose release. Kaufman and Bergman (1971), using cannulated conscious sheep, observed a net output of glucose by the kidneys under normal conditions of feeding, with no increase during fasting or acidosis.

The present study has been made using a similar preparation to that of Kaufman and Bergman (1971). However, observations were taken over much longer periods, and an automated method was used for continuous sampling and determination of arteriovenous differences of glucose for the kidney throughout the experiment. The

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influence of ammonium chloride acidosis on glucose production and renal function was determined, and the significance of these changes in relation to each other was assessed.

II. METHODS

Twelve adult Merino sheep (three ewes and nine wethers, aged between 2 and 3 years) were used for the determination of an arteriovenous difference in glucose concentration across the kidney. Seven of these were also used for a study of glucose production concurrently with renal function under normal and acidotic conditions. The animals had been trained to metabolism cages and were fed a daily ration of 750 g wheaten hay chaff and 250 g lucerne chaff. Water was provided *ad libitum.*

Following a 24-hr fast the sheep were anaesthetized with sodium thiopentone and Fluothane, and polyvinyl chloride cannulae (1·0 mm i.d., 1·5 mm o.d.; and 1·4 mm i.d., 1·9 mm o.d.) were surgically introduced into the left renal vein and the left ureter respectively. The cannulae were exteriorized via a sublumbar incision. Cannulae were also introduced into the left femoral artery and jugular vein. All vascular cannulae were flushed daily with heparin saline solution (100 units/ml).

On the day of the experiment a priming dose of *p*-aminohippuric acid (PAH) (8 mg/kg body weight) and inulin (40 mg/kg) was injected into the jugular vein, then a maintenance solution (1% PAH and 1% inulin in 0·9% NaCl solution) was infused over a 4-hr period at a rate of 1 ml/min. Urine was collected from the ureteric cannula over 30-min periods for a total time of 4 hr and blood samples were taken from the femoral artery and left renal vein 15 min from the start of each urine collection period. Haematocrits were determined on the arterial samples by centrifuging at 12,000 rev/min for 4 min, using a micro-haematocrit centrifuge. Each day before feeding the acid–base status of the sheep was determined on arterial samples collected anaerobically, stored on ice, and measured within 60 min. The method of Astrup (1957) and the nomogram of Siggard Andersen and Engel (1959) give a value for base excess (BE) which indicates the total excess of base. A negative BE value signifies a base deficit or an excess of non-volatile acid. During conditions of homeostasis a BE of zero is accepted as the normal value; for sheep's blood this is equivalent to 26 m-equiv. HCO_3^- per litre.

Metabolic acidosis was induced in the sheep by drenching with 20 g NH_4Cl daily in divided doses or by constant infusion of NH_4Cl into the rumen for 3–11 days. By these means acidosis was produced, leading in the majority of sheep to a maximum reduction in blood pH within 3 days.

Inulin was determined in plasma and urine by the method of Wilson *et al.* (1969) and PAH by the method of Harvey (1962). Inulin clearances (glomerular filtration rate) and PAH clearances were determined using the Fick principle. PAH clearance was corrected using the extraction ratio:

$$\frac{\text{(concentration in arterial plasma} - \text{concentration in renal venous plasma)}}{\text{concentration in arterial plasma}}$$

to give the effective renal plasma flow. This value was then multiplied by 100/(100 − haematocrit value) to give renal blood flow.

In order to obtain accurate arteriovenous differences for glucose a system was devised whereby blood was withdrawn through an Autoanalyser pump at a rate of 0·23 ml/min from both the femoral artery and the renal vein. The animal was heparinized with an initial injection of 10,000 i.u. followed by additional injections of 5,000 i.u. when required. A device was attached to the Autoanalyser sampler which enabled blood from artery and vein to be collected simultaneously into a pair of sample cups. After 3 min a switching mechanism shifted the outlet tubes to allow the filling of the next pair of cups. Meanwhile blood sampling for glucose determination was commenced from the first cup, and continued at 1·5-min intervals per sample. Blood glucose was measured by the automated glucose oxidase method of Cramp (1967). With this method a maximum of 50 pairs of arterial and venous blood samples were analysed for glucose at intervals throughout the 4-hr period. The blood glucose concentration for each animal was stable throughout this period. The data from all experiments yielded a coefficient of variation for glucose concentration of 1%. Results were analysed statistically using the Student *t*-test.

III. RESULTS

In 12 normal adult sheep a positive mean venous–arterial difference for glucose was established across the left kidney (310 μg/100 ml, S.E. 116). This is significantly different from zero ($P<0\cdot05$), indicating a net output of glucose by the kidney.

TABLE 1

NET GLUCOSE PRODUCTION BY ONE KIDNEY IN CONTROL SHEEP AND SHEEP MADE ACIDOTIC BY AMMONIUM CHLORIDE ADMINISTRATION

Experiment No.	Body weight (kg)	Control values			Values during acidosis		
		Glucose concn.* (μg/100 ml)	RBF† (ml min^{-1} kg^{-1})	Glucose output (μg min^{-1} kg^{-1})	Glucose concn.* (μg/100 ml)	RBF† (ml min^{-1} kg^{-1})	Glucose output (μg min^{-1} kg^{-1})
1	31·2	259	10·0	25·9	230	6·6	15·2
2	33·5	440	11·2	49·3	510	11·0	56·1
3	40·0	−176	4·7	−8·3	1176	3·4	40·0
4	36·5	200	7·0	14·0	969	3·9	37·8
5	34·6	77	8·5	6·5	1396	5·6	78·2
6	35·4	78	6·0	4·7	614	7·4	45·4
7	36·0	441	11·6	51·2	1888	8·0	151·0
Mean		188	8·43	20·5	969	6·55	60·5
S.E.‡					166	0·51	10·1
P					<0·02	<0·05	<0·05

* Venous–arterial difference.

† Renal blood flow.

‡ Standard error of difference (control–acidosis).

In seven of these animals glucose determinations were made concurrently with measurements of renal function and the data from these animals are presented in Table 1. The mean renal blood flow for the control group was 8·43 ml min^{-1} (kg body weight)$^{-1}$ and the venous–arterial difference was 188 μg/100 ml, giving a calculated net glucose output of 20 μg min^{-1} (kg body weight)$^{-1}$ for the one kidney. This value was not significantly different from zero.

In acidotic sheep the venous–arterial difference was increased to a mean value of 969 μg/100 ml and blood flow was reduced to a mean value of 6·55 ml min^{-1} (kg body weight)$^{-1}$, a statistically significant reduction ($P<0\cdot05$). The calculated net glucose output during acidosis was 60 μg min^{-1} kg^{-1} per kidney, and this increase in glucose output was significant ($P<0\cdot05$).

Acidosis produced by ammonium chloride ingestion led to a mean fall in blood pH from 7·45 to 7·20, which was associated with a shift in BE from 0 to −15 m-equiv/l, usually within 48–72 hr. In two of the seven sheep (experiments 1 and 5), in which ammonium chloride was administered for 11 and 5 days respectively, the venous–arterial glucose concentration increased by factors of 5 and 20 by the end of the period (Table 2). However, this was associated with inanition after 4 days,

so that a fasting state was thereafter superimposed on the experiment. In one animal (No. 1), in which there was only a very small change in acid–base status at 3 days, no change in the venous–arterial difference for glucose was observed. This could be responsible for the lack of increase in glucose output in this animal at 3 days (see Table 1).

TABLE 2

INFLUENCE OF CONTINUED DOSING WITH AMMONIUM CHLORIDE ON VENOUS–ARTERIAL GLUCOSE CONCENTRATION ACROSS THE KIDNEY AND ON ARTERIAL BLOOD pH

Experiment No.	Glucose concn.* (μg/100 ml)	Period (days)	Blood pH
1	259	0	7·49
	230	3	7·42
	800	7	7·41
	1060	11	7·29
5	77	0	7·45
	1400	3	7·17
	1500	5	7·18

* Venous–arterial difference.

TABLE 3

INFLUENCE OF CONTROL AND ACIDOTIC CONDITIONS ON RENAL FUNCTION PARAMETERS

GFR = glomerular filtration rate; ERPF = effective renal plasma flow. The ratio GFR : ERPF gives the filtration fraction

Experiment No.	Control values			Values during acidosis		
	GFR (ml/min)	ERPF (ml/min)	Filtration fraction	GFR (ml/min)	ERPF (ml/min)	Filtration fraction
1	30·4	246	0·12	28·4	162	0·18
2	30·4	297	0·10	62·5	292	0·21
3	15·0	162	0·09	14·8	117	0·13
4	35·0	202	0·17	26·4	112	0·24
5	—	232	—	22·6	152	0·15
6	31·0	167	0·19	28·4	207	0·14
7	49·6	310	0·16	21·0	219	0·10
Mean	31·9	231	0·14	29·2	180	0·16
S.E.*				5·66	19·2	0·027
P				n.s.	<0·05	n.s.

* Standard error of difference (control–acidosis).

Table 3 presents the data on the parameters of renal function in sheep when normal and acidotic. There was no significant change in mean glomerular filtration rate from control (31·9 ml/min) to the acidotic state (29·2 ml/min, S.E. of difference = 5·66). However, effective renal plasma flow showed a significant fall with the induction of acidosis, and in four experiments this led to an increase in the filtration fraction

(clearance of inulin ÷ corrected clearance of PAH), although the mean increase of the filtration fraction for the six animals was only 0·02.

IV. Discussion

The results presented here indicate that under normal conditions the net output of glucose by the kidneys of sheep is small in relation to the daily requirement of the animal. Adult sheep have an entry rate of about 120 g/day (Annison and White 1961), and the contribution of both kidneys is not more than about 2 g/day, i.e. less than 2%. Even during acidosis this value did not exceed 6 g/day.

Kaufman and Bergman (1971) reported values of 10–16% of daily requirements arising from the kidneys of normal sheep, a value much higher than our estimates. The venous – arterial differences for glucose concentrations were about 60% lower in our experiments, which would account for the smaller glucose production values. The reason for this difference is not apparent. However, with the use of the automated procedure and a large number of measurements over a long period a high degree of precision was possible. Moreover, with the greater degree of acidosis produced in our experimental animals, net glucose production was increased by about three times. By contrast Kaufman and Bergman (1971) were unable to detect any significant change in glucose output during acidosis.

An interesting observation in the present studies was the significant reduction in renal blood flow under conditions of acidosis. This was not influenced by the extraction ratio of PAH, which was unaffected by acidosis. A reduction in blood flow appeared in four of the five animals used by Kaufman and Bergman (1971) but the difference between groups was not significant. This reduction in renal blood flow was not accompanied by any significant change in glomerular filtration rate, which suggests that there may be a redistribution of blood flow, leading to a greater percentage of blood perfusing the active zone of the renal cortex. As there is a marked difference between cortex and medulla in terms of glucose metabolism (Lee and Peter 1969), it is possible that the change in glucose output could be accounted for in part by such a redistribution in blood flow.

However, the changes seen here are probably more indicative of changed intrarenal events associated with the control of acid–base homeostasis by the kidney. The contribution to overall glucose requirements in sheep is insignificant.

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