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Research Article

Using D-[1-^{14}C]glucose as a tracer, renal glucose utilization and production was measured in chronic metabolic acidosis and alkalosis in dog kidney in vivo. In six experiments in acidosis, mean total renal glucose production was 4.447±1.655 SE μmol/min and glucose utilization was 4.187±0.576 SE μmol/min. In five alkalotic experiments it was found that mean total glucose production was 12.227±2.026 SE μmol/min and glucose utilization was 18.186±2.054 SE μmol/min. Renal glucose utilization and production are therefore significantly higher in alkalosis than in acidosis in vivo. Since glucose production is maximal under conditions when glutamine extraction is minimal (i.e. alkalosis), it is apparent that in alkalosis glutamine is not a major precursor of glucose.

Glucose Utilization and Production by the Dog Kidney In Vivo in Metabolic Acidosis and Alkalosis

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ABSTRACT Using D-[1-^{14}C]glucose as a tracer, renal glucose utilization and production was measured in chronic metabolic acidosis and alkalosis in dog kidney in vivo. In six experiments in acidosis, mean total renal glucose production was 4.447±1.655 SE μmol/min and glucose utilization was 4.187±0.576 SE μmol/min. In five alkalotic experiments it was found that mean total glucose production was 12.227±2.026 SE μmol/min and glucose utilization was 18.186±2.054 SE μmol/min. Renal glucose utilization and production are therefore significantly higher in alkalosis than in acidosis in vivo. Since glucose production is maximal under conditions when glutamine extraction is minimal (i.e. alkalosis), it is apparent that in alkalosis glutamine is not a major precursor of glucose.

INTRODUCTION

In dog kidney cortical slices chronic metabolic acidosis increases and alkalosis decreases gluconeogenesis (1). In these studies glutamine was used as a substrate and a causal relationship between glucose production and glutamine utilization was suggested. In vivo, Steiner, Goodman, and Treble (2) also reported increased gluconeogenesis in the dog kidney in acidosis and again postulated that glutamine was the major source. In the subsequent in vivo studies of Roxe, Disalvo, and Balagura-Baruch (3), Churchill and Malvin (4), and Roxe (5) no significant renal gluconeogenesis could be demonstrated. Recently however Pitts, Pilkington, MacLeod, and Leal-Pinto (6) using [^{14}C]glutamine have shown substantially more incorporation of the label into glucose in acidosis than in alkalosis in dog kidney in vivo. It therefore seems possible that the discrepancy between the in vitro and in vivo data could be accounted for by glucose utilization in the intact kidney. In view of this possibility it remains unclear to what extent glucose production is accounted for by the carbon skeleton of glutamine. In addition, the effects of acid-base change on glucose production and utilization in vivo are not yet fully elucidated.

To this end we developed a sensitive enzymatic technique for measuring the specific activity of [1-^{14}C]glucose (7). It was thus possible to quantitate accurately glucose production and utilization under conditions of metabolic acid-base change. Our results indicate that renal glucose utilization and total renal glucose production are greater in chronic metabolic alkalosis than in chronic metabolic acidosis in vivo.

METHODS

Adult male mongrel dogs weighing 16–25 kg were used. Metabolic acidosis (six dogs) was induced by administration of 10 g of NH_4Cl in the food for 6 consecutive days. Metabolic alkalosis was induced by similar administration of 20 g $NaHCO_3$ for the same period. The dogs were fasted for 18 h before surgery. Anesthesia was induced with intravenous sodium pentobarbital (30 mg/kg) and when necessary small supplemental doses were administered intravenously during the experiment. An endotracheal tube was passed. Through a midline incision the left ureter was catheterized. A catheter filled with heparin-saline (20 IU/ml) was introduced into the inferior vena cava from the right femoral vein and its tip manipulated into the left renal vein. The left spermatic vein was ligated. For arterial blood sampling a catheter was introduced through the right femoral artery and its tip positioned in the aorta close to the origin of the renal artery. A catheter was also placed in the left femoral artery and connected via a pressure transducer to a cardiac pressure recorder for constant monitoring of the blood pressure.

30 min after the closure of the abdomen, a priming dose of [^{125}I]sodium iothalamate (The Radiochemical Centre, Amersham, England), 0.5 μCi/kg was administered intravenously followed by a sustaining dose of 0.0025 μCi × glomerular filtration rate (GFR)/min (where GFR estimated at 3 ml/kg). Simultaneously an intravenous dose of [1-^{14}C]glucose, 1.3 μCi/kg, was administered followed by a sustaining dose of 0.23 μCi/min via the recurrent tarsal vein using the Palmer slow injection apparatus. Preliminary studies indicated that, at these infusion rates, arterial [1-^{14}C]glucose remained remarkably stable over the period studied. An in-

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travenous infusion of normal saline was commenced through the cephalic vein and regulated in an attempt to achieve a satisfactory urine flow. After a 40 min equilibration period urine and arterial and renal venous bloods were sampled. Renal venous and arterial blood was withdrawn over a 10 min period through a Watson-Marlow flow inducer (Watson-Marlow Ltd., Falmouth, Cornwall, England), placed as closely as possible to the exposed tips of the catheters, at a rate of 2.0 ml/min. The blood was collected in heparinized universal containers containing 300 IU heparin (0.15 ml), packed in crushed ice. Urine was simultaneously collected. Between four and six consecutive 10-min renal clearance periods were observed. At the end of each clearance period the bloods were centrifuged for 5 min in a refrigerated centrifuge and the separated plasmas immediately stored at −12°C. Preliminary studies showed that no change could be detected in the glucose concentration of ice-cold blood over a 30 min period.

[^{125}I]iothalamate was counted in urine, arterial, and renal venous plasma using a Nuclear-Chicago gamma counter (model 4216 Nuclear-Chicago Corp., Des Plaines, Ill.). GFR was calculated from the iothalamate clearance (8). Renal plasma flow was also determined with [^{125}I]iothalamate using the Wolf (9) modification of the Fick principle.

A method was developed for the measurement of [1-^{14}C] glucose specific radioactivity in plasma (7). [1-^{14}C]glucose (specific activity 54.2 mCi/mmol) was obtained from the The Radiochemical Centre.The method is based on the use of three coupled enzyme reactions to convert glucose to ribulose-5-phosphate and carbon dioxide. The assays were carried out in counting vials using 0.1 ml of plasma and radioactive glucose determined by the disappearance of $^{14}CO_2$ after acidification. The ^{14}C was counted in toluene/Triton X-100 scintillation fluid (10) using a Packard liquid scintillation counter (model 3375, Packard Instrument Co., Downers Grove, Ill.) and the dpm calculated from the channels ratio. The method gives over 98% recoveries over the range of plasma glucose concentrations found in the dog. All determinations were carried out in triplicate. Total glucose was estimated in triplicate by the method of Slein (11). Plasma rather than whole blood was used based on the observations of McCann and Jude (12) that increments in glucose in the renal vein are largely in the plasma fraction. Arterial pH was measured using an Astrup (Radiometer) pH meter.

Net glucose production (micromoles per minute) was calculated as follows:

$$(RPF_v \cdot G_v - RPF_a \cdot G_a) \qquad (1)$$

Where RPF_v and RPF_a represent renal venous and renal arterial plasma flow rates (milliliters per minute), respectively and G_v and G_a represent renal venous and arterial glucose concentrations (micromoles per milliliter), respectively. Urine glucose concentration which was negligible under conditions of acidosis and alkalosis and was ignored in these calculations. Glucose breakdown (micromoles per minute) was calculated as follows:

$$RPF_a \cdot G_a \frac{(RPF_a \cdot g_a - RPF_v \cdot g_v)}{RPF_a \cdot g_a} \qquad (2)$$

Where g_a and g_v represent arterial and renal venous plasma [1-^{14}C]glucose activity respectively in dpm.

Total glucose production was calculated as the sum of the net glucose production and glucose breakdown—(1)+(2).

RESULTS

The initial activity of [1-^{14}C]glucose did not differ significantly between the two groups (Table I). The activity remained constant throughout the periods of observation. The regression coefficient of activity in dpm against time in minutes in alkalosis was 0.455±0.269 SE, not significantly different from zero ($t = 1.695$, $P > 0.1$). Similarly in acidosis the regression coefficient was 0.086±0.352 SE not significantly different from zero ($t = 0.244$ $P > 0.8$). This represents a statistically insignificant rise of less than 3% over 60 min in alkalosis and of less than 1% in acidosis.

Net arterial glucose production in the acidotic dogs 0.260±1.934 SE μmol did not differ significantly from zero ($P > 0.3$) shown in Table II. The results in the alkalotic dogs indicate a small but consistent net glucose extraction and the data suggests that the difference between the two groups may be statistically significant ($0.025 > P < 0.05$).

A highly significant difference in glucose breakdown was observed between the two groups of dogs. Whereas some breakdown was detectable in all the acidotic experiments, the extent was consistently less than was observed in alkalosis ($P < 0.001$).

With one exception (exp. 8) total glucose production was also consistently greater in alkalosis than in acidosis. The difference between the two groups was statistically significant ($P < 0.02$).

DISCUSSION

Conflicting evidence exists as to the effects of chronic metabolic acidosis on renal gluconeogenesis in vivo in the dog. The studies of Churchill and Malvin (4) emphasized the importance of continuous rather than midpoint sampling in studies of the renal handling of glu-

TABLE I

Arterial pH, Plasma [^{14}C]-Glucose Activity, and Renal Hemodynomics in Acidotic and Alkalotic Dogs

	Arterial pH	[^{14}C]-Glucose activity	GFR	Urine vol.	RPF
		dpm/100 μl plasma	*ml/min*	*ml/min*	*ml/min*
Alkalosis *(5)	7.54	814.0	27.20	1.61	138.11
±SE	0.01	73.74	2.663	0.467	19.92
Acidosis *(6)	7.21	845.3	20.06	1.73	110.66
±SE	0.03	61.64	3.461	0.242	19.87
P	0.01	NS	NS	NS	NS

* Number of dogs.
P, refers to the differences in the means in acidosis and in alkalosis.
NS, means not significant.
All data from one kidney.

TABLE II

The Effects of Acidosis and Alkalosis on Renal Glucose Production and Utilization In Vivo

Exp.	Arterial glucose per ml ±SE		Renal venous glucose per ml ±SE		Net glucose production	Glucose utilization	Total glucose production
	μmol	*dpm*	*μmol*	*dpm*	*μmol/min*	*μmol/min*	*μmol/min*
Alkalosis							
1	5.520±0.061	7,550±184	5.535±0.065	7,482±213	−6.217	+18.772	+12.555
3	5.836±0.133	6,917±187	5.804±0.115	6,817±165	−4.218	+12.728	+8.510
5	6.571±0.077	10,985±120	6.693±0.061	10,910±138	−7.144	+25.366	+18.222
7	6.365±0.073	8,062±70	6.435±0.082	7,951±101	−10.091	+17.218	+7.127
9	6.140±0.073	7,203±39	6.152±0.094	7,133±63	−2.126	+16.846	+14.720
Mean					−5.959	+18.186	+12.227
±SE					1.347	2.054	2.026
Acidosis							
2	4.087±0.112	7,380±225	4.285±0.132	7,580±71	−0.115	+2.859	+2.704
4	5.936±0.085	8,062±131	5.993±0.069	8,127±115	−3.647	+4.080	+0.433
6	6.143±0.070	11,037±126	6.329±0.081	11,245±140	+4.196	+2.681	+6.877
8	4.840±0.084	9,390±173	4.945±0.097	9,380±133	+7.738	+3.786	+11.524
10	6.227±0.161	7,920±105	6.307±0.161	8,013±92	−3.619	+6.139	+2.520
12	5.483±0.084	7,039±108	5.556±0.079	7,124±146	−2.953	+5.578	+2.625
Mean					+0.26	+4.187	4.447
±SE					1.934	0.576	1.655
P					<0.05	<0.001	<0.02

Each value represents the mean of four to six observations. Total glucose production is derived from the sum of net glucose production and glucose utilization.

P refers to the differences in the means in acidosis and in alkalosis.

cose. Using continuous arterial and renal venous sampling throughout the clearance period, we found no net production of glucose by the kidney in acidosis. These results confirm the observations of Churchill and Malvin (4), Roxe et al. (3), and Roxe (5).

Glucose utilization (micromoles per minute) was calculated from the arterial glucose concentration and the fractional change in the (renal venous—arterial) glucose radioactivity. Renal glucose utilization in acidosis was 4.187 μmol/min. By contrast, glucose utilization was significantly higher in alkalosis 18.186 μmol/min. Thus in alkalosis, there is a significant reduction in specific activity as compared with acidosis, meaning a greater disappearance of labeled carbon-1 as well as a greater appearance of unlabeled carbon-1, or in other words, a greater rate of exchange of isotope. Whether this is due to utilization of glucose-1 in one anatomic location and production of cold glucose in another or due to a greater rate of isotope exchange in one anatomic locus cannot be answered. The remarkably constant plasma [1-^{14}C] glucose concentrations however indicate a steady-state situation and make the former interpretation seem more likely. A possible criticism of this measurement of glucose utilization is that it represents a minimal value due to possible recycling of the carbon label. However, since [1-^{14}C]glucose was used the label would be completely lost via the hexose monophosphate (HMP) shunt and were recycling via the Krebs cycle to occur, only that fraction which was recycled to the carbon-1 position would be detected by the method.

The observations of Hostetler and Landau (13) that in kidney in vivo the contribution by the HMP shunt is small and of Dies and Lotspeich (14) that HMP shunt activity is less in alkalosis than in acidosis would suggest that the enhanced glucose utilization found in the present studies was due to glycolysis. This is supported by the findings of Gevers and Dowdle (15) who found increased glycolysis in renal slices in alkalosis, and by Scheuer and Berry (16) and Ui (17) who indicated that increased glycolysis in alkalosis was caused by activation of phosphofructokinase.

Total glucose production was greater in alkalosis than in acidosis and the difference was statistically significant ($t = 3.008$ $P < 0.02$). These results are in marked contrast to what has been found in vitro (1), since glucose production is maximal under conditions where glutamine extraction is minimal (i.e. alkalosis) and glucose production is minimal under conditions where glutamine extraction is maximal (i.e. acidosis).

Pitts et al. (6) reported that 20% of glutamine extracted in acidosis was converted to glucose. From the present results it appears possible that glutamine may be a major precursor of glucose in acidosis. In alkalosis, however, glucose production must derive from an alterna-

tive carbon source. Pitts et al. (6) have also shown that glutamine is a major metabolic fuel of the kidney in acidosis. From the present results it seems possible that glucose may be an impotrant renal energy source in alkalosis. Further studies are needed to measure the extent to which these results represent aerobic or anaerobic metabolism.

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