Increased basal gluconeogenesis in the aged rat

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Post-absorptive gluconeogenesis from lactate measured in vivo increases 3-fold in 24-month-old rats compared to 3-month-old animals. Fractional lactate turnover rates showed no significant differences between the two groups of animals. Lower plasma glucose concentrations and insulin-glucagon ratios may explain the increase in gluconeogenesis observed in aged rats.

Aging *Gluconeogenesis* *Lactate* *Insulin* *Glucagon*

1. INTRODUCTION

In the process of ageing the ability to utilize glucose progressively deteriorates throughout the adult life span [1–6]. However, it remains unknown whether the mechanism of the deterioration in glucose disposal is identical with, similar to, or entirely different from the mechanism underlying glucose intolerance in diabetes [6].

Two basic mechanisms that could explain the impaired glucose performance found in diabetic and/or aged animals have been reported to include (i) a decrease in glucose-induced insulin secretion from the β-cell [7] and (ii) decreased insulin sensitivity or state of insulin resistance in the tissues [8]. Both mechanisms contribute, at least in part, to the increased gluconeogenic capacity reported for the diabetic condition [7–10]. In [11] we found differences in the activities of enzymes linked to energy metabolism that suggested an increase in gluconeogenic capacity for the liver of the old rat. However, based on similar types of experiments it has been recently suggested that gluconeogenesis decreases in aged mice [12]. Therefore, we have re-evaluated the in vivo basal gluconeogenic capacity of the old rat. Our findings show that the gluconeogenic capacity of the old rat is increased. The results are discussed within the framework of the hormonal and metabolic changes that occur with ageing.

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2. EXPERIMENTAL

2.1. *Materials*

L-[U-^{14}C]Lactate was obtained from the Radiochemical Centre, Amersham. ^{125}I-labelled glucagon was obtained from the Nuclear Medical Laboratory, Dallas, TX. Rat insulin was purchased from Novo Research Institute, Bagsvaerd, Denmark. Antiglucagon antisera (30 kDa) were obtained from Dr R. Unger, Texas Health Science Center, Dallas, TX.

2.2. *Methods*

2.2.1. Animals

3- and 21-month-old male albino Wistar rats weighing 250 and 550 g, respectively, were fed on standard laboratory chow and water ad libitum. Rats were decapitated or injected intraperitoneally with the radioactive tracer 1 h after initiation of the diurnal cycle in our animal house (between 9 and 10 a.m.).

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2.2.2. Measurement of plasma substrates and hormones

6 ml blood were collected from the decapitated rats in 1 ml ice-cold normal saline solution containing 1% heparin. Glucose [13] and lactate [14] concentrations in blood, plasma, or in the chromatographic fractions were measured. For the hormone studies, 1 ml blood was collected in 1 ml ice-cold normal saline solution containing 10000 units Trasylol and 2.4 mg EDTA. Plasma was separated by centrifugation in the cold and samples stored at −70°C until assayed. Plasma insulin [15] and glucagon [16] concentrations were determined using rat insulin and porcine glucagon, respectively, as standards. Liver glycogen concentrations were also assayed [17] in freeze-clamped tissue [18].

2.2.3. Studies in vivo

Gluconeogenesis and $^{14}CO_2$ production were studied in non-anaesthetized rats after the administration of 10 μCi L-[U-^{14}C]lactate per 100 g body wt. as described [19]. The aminals were placed in a cage in which a slight negative air pressure was created by a low-performance vacuum pump. The air circulating through the cage was bubbled through 3.5 N KOH solution in which the $^{14}CO_2$ evoked by respiration of the animals was trapped. At the time intervals shown in fig.1, 50-μl samples of blood were collected from the tail vein and quickly deproteinized [19]. Blood glucose and lactate were separated with anion-exchange resin (Dowex AG 1-X8) as in [19]. The specific activity of each metabolite is expressed as dpm/μmol substrate. Fractional lactate turnover rates [19,20] and rates of gluconeogenesis from lactate [19,21] were calculated in the steady state for both blood glucose and lactate concentrations [19,21] (see fig.1). Statistical analysis was performed by Student's *t*-test. Linear regression correlation was applied to the plots with the aid of a digital computer using a FORTRAN IV program. *P* values of 0.05 or less were taken as significant, and the results expressed as means ± SE.

3. RESULTS AND DISCUSSION

3.1. *Rates of lactate turnover and gluconeogenesis*

Fig.1 shows the time courses of the logarithms of the specific activities of blood lactate and the specific activity itself of blood glucose after the administration of L-[U-^{14}C]lactate in one adult (3-month-old) and one old (21-month-old) rat. The radioactive lactate was administered when blood lactate and glucose concentrations were in the steady state [19] (see upper panels of fig.1). This figure represents one of 3 different experiments carried out with 3 young adults and 3 aged rats. The plot of log specific activity of blood lactate vs time (fig.1) showed slopes, *m*, of −0.0262 min^{-1} ($r = 0.9750$; $p < 0.001$) for the young adult rat and −0.0190 min^{-1} ($r = 0.9996$; $p < 0.001$) for the aged rat. The mean of the slopes for the 3 different ex-

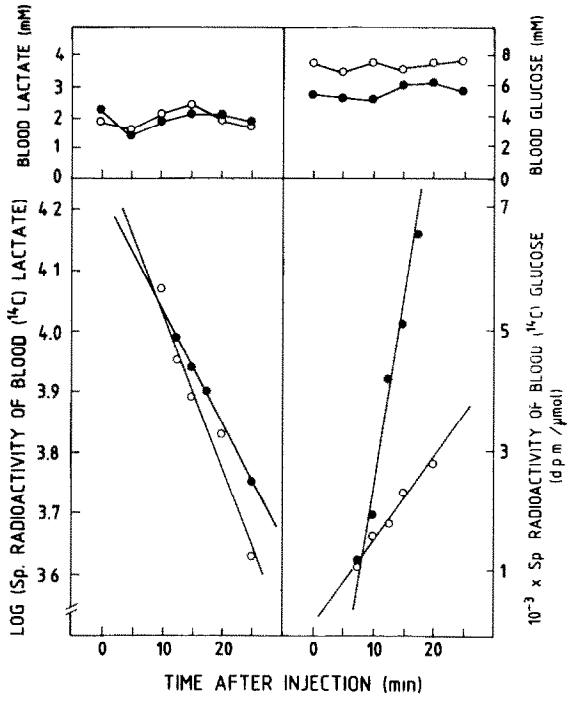

Fig.1. Logarithm of the specific activity of [^{14}C]lactate, specific activity of [^{14}C]glucose, and lactate and glucose concentrations in blood 25 min after the injection of [U-^{14}C]lactate in 3 (○) and 21 (●) month old rats. Rats were injected intraperitoneally with 10 μCi L-[U-^{14}C]lactate per 100 g body wt between 9 and 10 a.m. Every 2.5 min after the injection of the tracer, 50-μl samples of blood were collected from the tail vein. Blood glucose and lactate were separated as described in Section 2.2, the specific activity of each metabolite being represented as dpm/μmol. The results depicted are a typical plot of data obtained for both groups of experimental animals. The results of this plot and of two other experiments showing similar patterns are collected in table 1.

periments performed was $-0.0264 \pm 0.0048\ min^{-1}$ for aged rats, the respective correlation coefficients for the 3 different slopes being in the ranges 0.9589–0.9768 and 0.9780–0.9996. The significance of each plot was always higher than $p < 0.01$. The slopes for the plot of specific activity of blood glucose vs time (fig.1) were $132\ dpm \cdot \mu mol^{-1} \cdot min^{-1}$ ($r = 0.9841$; $p < 0.001$) for the young adult rat and $610\ dpm \cdot \mu mol^{-1} \cdot min^{-1}$ ($r = 0.9933$; $p < 0.001$) for the aged rat. The mean of the slopes for the 3 different experiments performed was 163 ± 23 and $741 \pm 66\ dpm \cdot \mu mol^{-1} \cdot min^{-1}$ for young adult and aged rats, respectively, the corresponding correlation coefficients for the 3 different slopes being in the range 0.9448–0.9841 and 0.9492–0.9999. The significance of each plot was always higher than $p < 0.01$.

By using the results depicted in fig.1 and those obtained in two similar experiments, the fractional turnover rate of blood lactate and the rate of lactate incorporation into blood glucose were calculated. The results are summarized in table 1. Lactate turnover rates showed no significant differences between the two groups of animals studied. Similarly, blood lactate concentrations (table 2) showed no changes with ageing. These results indicate that no significant differences should be expected to occur in the rates of lactate production by the tissues of the aged rat. However, the rates of lactate incorporation into blood glucose showed a 3-fold increase in old rats compared to young adults (table 1).

Table 1

Fractional turnover rates of lactate and rates of lactate incorporation into blood glucose in 3- and 21-month-old rats

Parameter	Age of rat	
	3 months	21 months
Lactate fractional turnover rate (min^{-1})	-0.060 ± 0.011	-0.046 ± 0.004
Rate of [^{14}C]lactate incorporation into blood glucose (μmol/min per 100 g body wt)	7.42 ± 1.72	22.34 ± 3.25*

*$p < 0.005$

For experimental details see legend to fig.1 and section 2.2. The results are means ± SE ($n = 3$). Values that are significantly different by Student's t-test from those for 3-month-old rats are shown

Table 2

Liver glycogen, plasma metabolites and plasma insulin and glucagon concentrations in 3- and 21-month-old rats

	Age of rat	
	3 months	21 months
Liver glycogen (μmol/g wet wt)	250 ± 25	223 ± 21
Plasma glucose (mM)	7.3 ± 0.4	5.5 ± 0.3**
Plasma lactate (mM)	2.8 ± 0.3	2.9 ± 0.2
Plasma insulin (μU/ml)	41.5 ± 1.2	46.7 ± 8.5
Plasma glucagon (ng/ml)	0.65 ± 0.05	1.13 ± 0.29*

*$p < 0.1$
**$p < 0.005$

Rats were decapitated between 9 and 10 a.m., liver and blood collected and the metabolites and hormones assayed as described in section 2.2. Results are means ± SE ($n = 3$–8). Values that are significantly different by Student's t-test from those for 3-month-old rats are shown

Because the rates of lactate production are not significantly altered by ageing whereas its metabolic fate by the Cori cycle is increased 3-fold, it is reasonable to suggest that ageing may impair lactate utilization by other pathways and/or tissues of the old rat, resulting in greater utilization of lactate for glucose production. In fact, the 'estimated' rates of lactate incorporation into CO_2 in old rats were significantly lower than in young adults (1.36 ± 0.16 and $0.18 \pm 0.04\ \mu mol$ lactate/min per 100 g body wt, for young adult and aged rats, respectively). This 10-fold difference in the rates of lactate oxidation might have been overestimated because no correction for HCO_3^- body pool size of the animals was taken into consideration and the appearance of radioactive CO_2 could result from both lactate oxidation and lactate-derived glucose oxidation. However, the results clearly indicate a qualitative decrease in lactate oxidation in the aged rat. A decrease in oxidative metabolism in old rat brain has also been reported [22]. Taken together, these results indicate that in old animals, a higher

fraction of the available lactate is channelled via gluconeogenesis. This would prevent lactate accumulation in the blood (or any decrease in its rate of turnover) in agreement with the result reported in tables 1 and 2.

3.2. *Hormonal and metabolic changes*

The hormonal and metabolic changes observed in our colony of old rats are summarized in table 2. Post-absorptive plasma glucose concentrations are 25% lower in old rats than in adults. In the rat, the effects of ageing on plasma glucose concentrations remain controversial. Conflicting reports have varied from changes similar to those we have reported [12,13], no changes [24,25] or even an increase in plasma glucose concentration accompanying ageing [26]. The differences in glycogen concentrations between adult and old liver reported to date vary from no significant changes (present study, table 2) to significantly different changes [27,28], in some reports being 60% lower than the concentrations found in adult rats [29].

The hormonal profile favours an increase in gluconeogenesis in old rats. Plasma insulin concentrations (table 2) show a slight increase which is not statistically significant in old rats compared to adults. However, it is generally accepted that ageing is accompanied by a state of insulin resistance in tissues of the old animal [30,31]. Plasma glucagon concentrations significantly increase in old rats (table 2). This increase results in lower insulin/glucagon ratios for the old rat compared to adults (64 and 41 for adult and old rats, respectively). Lower insulin/glucagon ratios favour an increase in gluconeogenesis, since it has been reported that changes in the ratio of the two hormones are more important in terms of regulation of the pathway than the changes in the concentrations of the individual hormones [32].

In conclusion, we report a 3-fold increase in the post-absorptive rates of gluconeogenesis from lactate in the old rat, concurrent with lower plasma glucose concentrations and lower plasma insulin/glucagon ratios. The increased gluconeogenic capacity of the old rat may be due to decreased utilization of the circulating lactate by other tissues.

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