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THE TURNOVER OF BLOOD GLUCOSE AND PLASMA FREE FATTY ACIDS IN THE RAT AFTER ACUTE CARBON DISULPHIDE INTOXICATION

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Abstract—The turnover of plasma free fatty acids and blood glucose has been measured in starved rats exposed to inhalation of carbon disulphide (2 mg/l) for 15 hr overnight. Measurements were made following single intravenous injection of [U-^{14}C]palmitic acid or of [5-^{3}H]glucose and [U-^{14}C]glucose in groups of exposed and control rats. CS_2 intoxication leads to a small but significant decrease in the rate of utilization of plasma free fatty acids and it is suggested that decreased availability of plasma free fatty acids leads to the increased catabolism of amino acids and the relatively large increase in urea production which is seen in starved CS_2-treated rats. Increased urea production is not associated with an increase in the absolute rate of production and utilization of blood glucose as measured using [5-^{3}H]glucose. There is an increase in the fractional turnover of blood glucose, but this is accompanied by a small hypoglycaemia in starved CS_2-treated rats. The fraction of glucose carbon which is recycled is unchanged in treated rats. A comparison has been made between fed and 24 hr-starved rats in their response to exposure to CS_2 (2 mg/l) for 4 hr. The results indicate that in contrast to starved rats, plasma free fatty acid utilization increases when fed rats are exposed to CS_2 and there is no increase in urea production. Inter-relationships between these effects are discussed.

When starved rats are exposed to inhalation of carbon disulphide (15 hr overnight at 2 mg/l), plasma urea concentration and the rate of urea excretion increase by about 50% [1]. At the same time there are small but statistically significant decreases, relative to corresponding starved controls, in the concentrations of both plasma free fatty acids (FFA) and glucose [1, 2]. It was suggested [1] that there may be an increased rate of utilization of glucose in starved rats after CS_2-intoxication, and that gluconeogenesis from amino acid precursors might be stimulated. These suggestions however were based on simple observations of changes in the concentrations of plasma constituents and it was proposed that they should be tested by more direct measurement of the turnover of blood glucose.

To clarify the interrelationships between glucose, FFA metabolism, and the rate of urea production in starved rats after acute CS_2 intoxication, therefore, measurements of rates of utilization of plasma FFA and blood glucose are now presented. It is shown that the increased catabolism of proteins and amino acids indicated by increased urea production after CS_2 intoxication [1] is associated with a small but significant decrease in the rate of utilization of plasma FFA, the major fuel in starved rats. Despite an increase in the potential supply of gluconeogenic precursors however the rate of gluconeogenesis is not increased in starved rats after CS_2-intoxication. There is an increase in the metabolic clearance rate (or fractional turnover) of blood glucose after CS_2-intoxication, which leads to a small hypoglycaemic effect as the absolute rate of production is unaltered.

Since the net breakdown of protein in a given tissue may depend on the supply to that tissue of alternative (non protein) substrates, such as fatty acids and glucose [3], it was of interest to compare the responses of fed and starved rats to exposure to CS_2. This has been done by using a short (4 hr) exposure to CS_2 and it is shown that the effect of CS_2 on plasma FFA concentration is dependent on nutritional state, and not on length of exposure as was previously suggested [1]. Corresponding differences between fed and starved rats in the response of plasma urea concentration to CS_2 intoxication are also shown. The results obtained are discussed in terms of nitrogen loss and substrate supply in CS_2-intoxicated rats.

MATERIALS AND METHODS

Treatment of animals. Male albino rats of the Porton strain, weighing 180–220 g in the fed state, were exposed to 2 mg CS_2/l of air in a vertical constant flow exposure chamber [4]. Control rats were kept in similar chambers without CS_2. There was no access to food or water in the chambers. Results are also quoted from previous experiments [1] in which heavier rats (200–250 g) of the same strain were treated similarly.

Two periods of exposure were used —(1) Rats were exposed for 15 hr overnight (18.30 hr–09.30 hr), food having been removed from the rats at 09.30 hr on the day exposure was started. These rats were used for the measurement of the turnover of blood glucose and plasma FFA (see below). (2) Fed rats or rats starved for 24 hr were exposed for 4 hr (10.00 hr–14.00 hr). Within 1 hr of the end of exposure, these rats were anaesthetized with diethylether and decapitated. FFA and urea were assayed in plasma samples as previously described [1].

Turnover of plasma FFA. The rate of utilization (or rate of irreversible disposal) of plasma FFA was measured in groups of CS_2-treated and control rats under ether anaesthesia according to methods described in detail elsewhere [5] which are summarised here. All experiments were carried out at room temperature (18–20°).

Immediately prior to experiments [1-^{14}C]palmitic acid (The Radiochemical Centre, Amersham) was complexed to serum obtained from donor rats. Four groups of experimental rats were then studied. Rats in one CS_2-treated group and in one control group were injected via a tail vein with [1-^{14}C]palmitic acid (25 μCi) in serum (0.2 ml) obtained from CS_2-treated donor rats. Similarly a treated and a control group were injected with tracer in serum from control rats. At each of 5 times (8 sec, 26 sec, 47 sec, 1 min 45 sec, 3 min) after injection 4 rats from each group were decapitated, and blood collected in heparin. Plasma samples were analysed for [^{14}C]FFA and FFA concentrations, and data normalized to a constant quantity (D dpm) of tracer injected per 100 g body wt.

Subsequent calculations were based on the quantity of FFA label per ml of plasma, rather than per total plasma volume as in previous work [5]. The areas to infinity under the quantity of label-time curves (A dpm.min.ml^{-1}) were calculated and estimates made of the standard error on the areas as described by Heath and Cunningham [6]. The donor group had no significant effect on area, and data were therefore pooled into one group of treated and one group of control rats.

A metabolic clearance rate (MCR, ml plasma.min^{-1}.100 g^{-1}) was calculated from the relationship MCR = D/A [6], assuming MCR to remain constant in each rat over the experimental period [5]. The rate of utilization (μequivs FFA.min^{-1}.100 g^{-1}) was calculated as the product of MCR and the mean plasma FFA concentration in each group of rats.

Turnover of blood glucose. D-[5-^{3}H]Glucose and D-[U-^{14}C]glucose were obtained from Radiochemical Centre, Amersham. Estimates of the rate of utilization of blood glucose and of the degree of glucose carbon recycling were made in 24 hr starved rats following a single intravenous injection of both tracers and were based on the work of Heath *et al.* [7, 8] and of Katz *et al.* [9, 10].

Conscious rats were injected via a tail vein with [5-^{3}H]glucose (25 μCi) and [U-^{14}C]glucose (5 μCi) in saline (0.2 ml). At each of four times after injection (Table 1) groups of 4 or 5 rats were decapitated and blood collected in heparin. Glucose concentrations and glucose specific radioactivities in samples (1 ml) of whole blood were measured after paper chromatography as described by Frayn [11], allowing calculation of the quantity of each isotope in glucose per ml of blood. The areas to infinity under quantity of label-time curves were calculated [6] and are designated A_H in the case of [5-^{3}H]glucose and A_C in that of [U-^{14}C]glucose.

Table 1. Sampling times and blood glucose concentrations during measurements of glucose turnover

Time (min)	Blood Glucose Concentration (mg/ml) Control	CS_2-treated
6	0.953 ± 0.082 (5)	0.798 ± 0.028 (4)
23	0.849 ± 0.029 (4)	0.760 ± 0.063 (5)
50	0.754 ± 0.035 (4)	0.647 ± 0.027 (5)
120	0.790 ± 0.084 (5)	0.696 ± 0.043 (4)

All rats had been starved for 24 hr prior to the experiments.

Experimental procedure is described in Methods. Results are expressed as mean ± S.E.M. with number of rats in parentheses.

Blood glucose concentrations over the experimental period are shown in Table 1. These show small deviations from the steady state. Even substantial deviations from steady state do not invalidate the calculation of mean MCR from the simple relationship MCR = D/A_H although rates of utilization cannot be directly calculated [8]. It was assumed that MCR was independent of blood glucose concentration over the observed range and the rate of utilization of blood glucose at any one time was calculated as the product of the mean MCR (from [5-^{3}H]glucose) and blood glucose concentration at that time. An estimate of the extent of glucose carbon label recycling was calculated from the ratio $(A_C - A_H)/A_C$ [9].

Standard errors on rates of utilization. Rates of utilization are given by the product of concentration and MCR. In order to calculate standard errors on rates of utilization, the square of the coefficient of error was equated with the sum of the squares of the coefficients of error on concentration and MCR, where coefficient of error is defined as (S.E.M./mean). A more detailed analysis of the results (not presented) suggests an inverse correlation between MCR and blood glucose concentration within a group. The above calculation therefore tends to overestimate the true error on rate. Such overestimation would not

Table 2. Turnover of plasma FFA in starved rats after exposure to CS_2

	Plasma FFA concentration (μequiv./ml)	Metabolic clearance rate (ml/min/100 g)	Rate of utilization (μequiv./min/100 g)
Control	0.801 ± 0.023	6.15 ± 0.201	4.93 ± 0.214
CS_2-Treated	0.696 ± 0.018	6.11 ± 0.265	4.25 ± 0.215
	P < 0.001	N.S.	P < 0.05

Rats were exposed in the presence or absence of CS_2 (2 mg/l) for 15 hr overnight without access to food. FFA turnover was measured as described in Methods. Results are expressed as mean ± S.E.M. The significance of differences between means was assessed by Students *t*-test. Data was pooled from 40 rats in each group as described in Methods.

Table 3. Turnover of blood glucose in starved rats after exposure to CS_2

		Control	CS_2-Treated	
MCR ([5-^{3}H]glucose) (ml/min/100 g)		1.74 ± 0.08	1.97 ± 0.06	$P < 0.05$
MCR ([U-^{14}C]glucose) (ml/min/100 g)		1.28 ± 0.05	1.51 ± 0.05	$P < 0.01$
% Recycling		26.5 ± 1.4	23.3 ± 1.1	$0.05 < P < 0.1$
	Time (min)			
Rate of utilization (mg/min/100 g)	6	1.66 ± 0.16	1.57 ± 0.07	NS
	23	1.48 ± 0.09	1.50 ± 0.13	NS
	50	1.31 ± 0.09	1.27 ± 0.07	NS
	120	1.38 ± 0.16	1.37 ± 0.09	NS

Measurements were made as described in Methods on rats which had been starved for 24 hr and had been exposed to CS_2 (2 mg/l) for 15 hr overnight. MCR = metabolic clearance rate. Rates of utilization are calculated from data in Table 1 and MCR obtained using [5-^{3}H]glucose. Results are expressed as mean ± S.E.M. The significance of differences between mean was assessed using Student *t*-test. The number of rats used is given in Table 1.

affect the conclusions reached. The significance of differences between means was assessed using Students *t*-test, for (*n*-2) degrees of freedom, where *n* is the total number of rats compared.

RESULTS

(a) *The turnover of plasma FFA in starved rats after CS_2-intoxication.* The rate of utilization of plasma FFA was measured in starved rats exposed to CS_2 overnight and in corresponding controls as described in the Methods section. In agreement with previous results [1], the plasma FFA concentration was significantly lower in treated rats (Table 2). The metabolic clearance rate was the same in both groups of rats. Thus there was a small (15 per cent) but significant decrease in the rate of FFA utilization after CS_2-intoxication (Table 2). These results indicate that acute CS_2-intoxication reduces the rate of production of plasma FFA and that the rate of utilization declines in proportion to the decrease in plasma FFA concentration.

(b) *The turnover of blood glucose in starved rats after CS_2-intoxication.* Apparent metabolic clearance rates of blood glucose were measured in starved CS_2-treated rats and in starved controls by using [5-^{3}H]glucose and [U-^{14}C]glucose and are shown in Table 3. The metabolic clearance rate measured by either tracer was significantly greater in CS_2-treated rats than in controls. Blood glucose concentrations throughout the experiment (Table 1) were consistently lower in CS_2-treated rats than in controls. A significant decrease in blood glucose concentration after exposure to CS_2 for the same period has also been reported previously [1]. The rates of utilization of blood glucose presented in Table 3 were calculated by using the mean metabolic clearance rate of [5-^{3}H]glucose (see Methods section) and are subject to a qualification given below. There was no difference in the rates of utilization between the groups throughout the experimental period.

The rate of utilization equals the rate of replacement in the steady state. Data in Table 1 suggest that a steady state was approached at the end of the experiment. These results therefore indicate that the rate of gluconeogenesis is unchanged in CS_2 treated starved rats. Estimates of the extent of glucose carbon recycling tended to be lower in CS_2-treated rats (Table 3) but this effect was not statistically significant.

The possibility has not been excluded that the increase in the metabolic clearance rate as measured by the irreversible loss of [5-^{3}H]glucose in CS_2-treated rats is due to an increase in futile cycling at the triose phosphate stage of glycolysis [12], rather than an increased clearance of glucose from blood. However, Katz *et al.* [10] point out that it is likely that the contribution of futile cycling to the irreversible loss of [5-^{3}H]glucose in starved rats *in vivo* is quite small. Furthermore, if increased futile cycling were the case, then the lower blood glucose concentration of CS_2 treated rats would indicate a decreased rate of gluconeogenesis. The hypothesis that is being tested is that there is an increased rate of gluconeogenesis and this is clearly disproved by the present results.

(c) *Plasma FFA and urea concentrations in fed and starved rats after CS_2 intoxication.* The decreased rate of utilization of plasma FFA in rats exposed to CS_2 overnight (15 hr) without access to food is accompanied by an increased rate of production of urea and a proportional increase in plasma urea concentration relative to corresponding starved controls. The results presented in Table 4 show that a qualitatively similar but smaller response occurs in rats exposed for a shorter period (4 hr) if they have been previously starved for 24 hr. However, exposure of fed rats for 4 hr results in a marked increase in plasma FFA concentration and no significant change in urea concentration. Differences between a short and long exposure reported previously [1] are therefore due to nutritional state and not length of exposure.

DISCUSSION

The rate of utilization of plasma FFA is proportional to their concentration in plasma over a wide range [5]. Furthermore exposure to CS_2 has no effect on the metabolic clearance rate of plasma FFA in starved rats. It is therefore reasonable to assume that

Table 4. FFA and urea concentrations in plasma of fed and starved rats after exposure to CS_2 (2 mg/l) for 4 hr

Rats		FFA (μequivs/ml plasma)		Urea (mM)	
Fed	Control	0.481 ± 0.024 (24)	P < 0.02	5.28 ± 0.27 (12)	N.S.
	CS_2-treated	0.600 ± 0.038 (24)		5.62 ± 0.29 (12)	
24 hr-starved	Control	0.974 ± 0.03 (10)	P < 0.01	5.61 ± 0.17 (18)	P < 0.001
	CS_2-treated	0.777 ± 0.039 (8)		7.18 ± 0.21 (16)	

Food was removed at 09.30 hr either on the same day as exposure or on the previous day. Rats were exposed from 10.00 hr to 14.00 hr as described in the Methods section. "Fed" rats were in a post-absorptive state when blood samples were obtained at the end of exposure. Results are presented as mean ± S.E.M. with number of rats in parentheses. The data on FFA concentrations in fed rats are taken from Cunningham (1975) [1] and are presented here to facilitate comparison with present work.

the increased FFA concentration in fed rats exposed to CS_2 indicates an increased rate of production and utilization. Freundt and Kurzinger [13] report increased oxygen consumption in rats given free access to food during exposure to CS_2 at concentrations up to 1.2 mg/l. The present results suggest that this increase in oxygen consumption is met by increased mobilization of FFA.

Whereas in fed control rats plasma FFA utilization is at a low level and increases readily in response to various stressful stimuli, that in starved rats is already high, but is decreased on exposure to CS_2. The reasons for this difference between fed and starved rats are not known. It may be noted that various other toxic effects of CS_2 are modified by nutritional state, for example, effects on the heart and liver [14, 15] and on catecholamine metabolism in adrenals [16]. Starvation may play a dual role in the toxicity, affecting both the metabolism of CS_2 [17] prior to the toxic lesions, and also in determining the possible adaptive responses of the rat subsequent to the lesions.

Although the decrease in the concentration of plasma FFA was small in starved CS_2-treated rats, the decrease was sustained and was observable after 4 hr exposure and after 15 hr exposure. A decreased rate of utilization may involve changes in the rate of esterification or of oxidation of fatty acids. A disturbance in esterification is suggested by a fall in plasma esterified fatty acid concentration after exposure to CS_2 [1]. A decreased rate of oxidation of fatty acids might be associated with a decreased oxygen consumption or increased oxidation of alternative fuels. The former is unlikely in view of the increase in oxygen consumption that occurs in fed rats after CS_2-intoxication. With respect to the latter possibility, since fatty acids constitute the major fuel in starved rats [18], a small percentage decrease in their oxidation rate would be compensated only by a larger percentage increase in the oxidation of other substrates. Thus the increased rate of urea excretion which accompanies decreased fatty acid utilization in starved rats, suggests the possibility that increased amino acid catabolism occurs in response to a decreased availability of fatty acids. Such a response occurs after administration of various antilipolytic agents to starved rats, for example nicotinic acid [19], nicotinamide [20] and 3-5-dimethylisoxazole [21]. In these cases the decrease in plasma FFA concentration and the increase in urea production are larger than in the case of CS_2 but the proportionality between the responses is similar.

The size of the increase in urea excretion after CS_2 treatment [1] in starved rats suggests that it is due to catabolism of amino acids derived from skeletal muscle protein. The transport of amino nitrogen from the periphery to the liver is intimately related to gluconeogenesis and the recycling of glucose carbon [22]. In CS_2-intoxicated rats therefore increased transport of amino nitrogen may involve either an increase in the production of amino acids from glucose derived carbon, or an increase in the export from the extrahepatic tissues of amino acids derived directly from protein breakdown. Quantification of these processes using tracer techniques alone is however difficult because of isotopic dilution in both liver and the periphery. An estimation may be made of the fraction of glucose derived from amino acids assuming 3.9 g of glucose to be generated per 1 g of urea nitrogen excreted [23]. In previous experiments [1] urea excretion was measured in CS_2-treated and control rats following an intragastric water load, and was 2.76 and 4.14 μmoles/min/100 g in control and treated rats respectively. These figures correspond to 22% and 33% of the glucose produced in control and CS_2-treated rats in the present experiments. This increase in the provision of amino acids for gluconeogenesis would take the form of an absolute increase in the rate of gluconeogenesis of the order of 10–15%. There was no evidence for any such increase although the results presented are not precise enough to rule out the possibility (Table 3). Alternatively the increased transport of amino groups from the periphery to the liver could have involved the *in situ* oxidation of carbon skeletons derived from protein breakdown in muscle and an increase in the fraction of glucose recycled via amino acids, for example by the amination of glucose derived pyruvate [22]. In this case the present results are consistent with a corresponding diminution in the recycling of glucose via other intermediates, e.g. lactate since there was no increase in the total rate of glucose carbon recycling. Both these possibilities are subject to further investigation.

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