EFFECTS OF HALOTHANE ON GLUCOSE METABOLISM AFTER INJURY IN THE RAT

D. F. HEATH, K. N. FRAYN AND J. G. ROSE

SUMMARY

The effects of halothane anaesthesia on glucose metabolism have been investigated in rats after a non-lethal scald injury. Anaesthesia was induced about 70 min after injury. Glucose metabolism was studied at two stages: during and shortly after induction, and about 2 h after induction. Comparisons were made with conscious rats at the corresponding times after injury. All rats were in an ambient temperature of 30 °C. During and shortly after the induction of anaesthesia, halothane caused a rapid increase in plasma glucose concentration, which by 30 min had begun to return to the values in injured controls; thus glucose production and utilization were increased. Insulin concentrations were increased also. However, after 2 h exposure halothane had decreased glucose production and utilization, as determined with [5-3H]- and [U-14C]-glucose, increased plasma concentrations of insulin and decreased liver concentrations of glycogen, that is it had exacerbated well-known effects of injury in the rat, including insulin resistance. Hyperglycaemia was not increased.

In the uninjured post-absorptive rat the effects of halothane on glucose metabolism (Biebuyck and Lund, 1974; Heath, Frayn and Rose, 1977a) are qualitatively similar to those of the "ebb" phase (Cuthbertson, 1942) after severe injury (Heath, 1973a; Frayn, 1975): an initial rapid mobilization of liver glycogen followed by impaired glucose utilization and decreased sensitivity to insulin. Since many injured patients have to undergo surgery, it is important to know whether halothane exacerbates the effects of injury on carbohydrate metabolism. This possibility has received little attention, although it is well known that carbohydrate metabolism is affected by anaesthetics (Brunner, Cheng and Berman, 1975). Therefore, we have carried out a preliminary investigation on rats injured by a severe but non-lethal scald, and have found that halothane exacerbated certain of the effects markedly.

MATERIALS AND METHODS

Animals and procedures

Male, Porton-Wistar albino rats weighing 239–253 g were fed on diet MRC 41B and maintained in a room at an ambient temperature of 20 °C lit from 07.00 to 19.00 hours. Food was removed on the day of the experiment.

About 3 h later the rats were anaesthetized with ether and given a full-thickness dorsal scald of 20% of the body surface (Arturson, 1961). Anaesthesia lasted 5–8 min. A tail vein of those rats to be injected was cannulated while the animals were still anaesthetized, while in those undergoing blood sampling during induction, a jugular vein was cannulated 4–8 days previously.

Immediately after the injury the rats were moved to a room at 29–31 °C ambient—the thermoneutral zone. This was necessary to keep their core temperatures near normal, since both injury and halothane anaesthesia reduce heat production to basal (Stoner, 1969; Heath and Rose, 1976). The core temperatures in rats used for the isotope experiments under halothane were in the range 36.0–38.5 °C.

Control animals were studied in the 30 °C room. Rats to be given halothane were transferred rapidly 70 min after injury to an open laboratory and placed, for the duration of the experiment, in chambers (desiccators) warmed to about 30 °C through which the anaesthetic mixture was passed (Heath and Rose, 1976). During the induction of anaesthesia halothane was administered in concentrations of 2.0–2.5% (v/v). Later the concentrations were decreased over 1 h to 1.0–1.2% (v/v), and these were sufficient to maintain Stage III anaesthesia. The oxygen content of the mixture was about 25% (v/v), as required to maintain normal PaO₂ values (Heath and Rose, 1976).
Glucose utilization rates

Each rat received, via the tail cannula, 6 μCi of [U-14C]-glucose, 35 μCi of [5-3H]-glucose and 350 i.u. of heparin in 0.2 ml of 0.9% sodium chloride, followed immediately by 0.2 ml of 0.9% sodium chloride to clear the cannula. The rats were decapitated at set times after injection, and haematocrit values, glucose concentrations and glucose specific radioactivities were determined. The four sampling times were 6, 30, 65 and 140 min after injection, and represented a compromise between the rather longer ideal times, calculated as described by Heath and Cunningham (1975), and the need to finish the experiment while the rats were still in the acute "ebb" phase.

Rates and rate coefficients (equivalent to metabolic clearance rates in the units used) were calculated. Since the plasma glucose concentration in different rats covered a wide range, the regression of the rates and rate coefficients on plasma glucose concentration could be calculated with standard error bands, and results are presented in this form. The range of concentrations shown embraces about 90% of the observed values. Degrees of freedom for injured rats exceeded 15 in every case.

Details of the method, essentially that of Heath and Corney (1973), have been deposited with the Royal Society of Medicine, Document No. BJA 78/1. Copies may be obtained also from the authors.

Analytical methods

Microhaematocrit values were corrected for entrained plasma (Heath, 1973b). Plasma insulin concentrations were determined as described by Heath, Frayn and Rose (1977b) using rat insulin standard (kindly provided by Dr J. Schlichtkrull, Novo Research Institute, Copenhagen), and liver glycogen concentrations (Heath, Frayn and Rose, 1977a) on 0.5-g samples from two different lobes of each liver. In non-isotopic experiments plasma glucose concentrations were determined using a hexokinase method (Boehringer Corporation (London) Ltd, Lewes, East Sussex). In isotope experiments glucose specific radioactivities and concentrations were determined on whole blood (Frayn, 1976a; Heath, Frayn and Rose, 1977b) and values in plasma calculated (Heath, 1973b). These calculations required values of the ratios of concentrations and of specific radioactivities in red cells and plasma at the various sampling times. For conscious injured rats previous values (Heath, 1973b) were interpolated. It was shown that, in injured rats under halothane, the ratios of concentrations were very similar. The ratios of specific radioactivities were assumed to be the same as in conscious injured rats, since the calculations are rather insensitive to the values used.

The statistical procedures used are described by Armitage (1971).

RESULTS

General responses to injury

Plasma glucose concentrations were increased in all the injured rats, except in a few animals in which, at more than 4 h after injury, values were in the upper half of the normal range. Haematocrit values were determined in injured rats during isotope experiments and were all greater than normal (51.2 ± 1.8%, mean ± SD, n = 70). These findings are characteristic of severe non-haemorrhagic injury in the rat.

Glucose metabolism during and shortly after induction

There was a very rapid further increase in the plasma glucose concentration during induction (fig. 1B). Two other sets of rats were studied to exclude the possibility that the effects noted at the time of induction were attributable to the transfer of the rats from the 30°C room to the anaesthetic chambers. Three patterns were observed. In controls remaining in the hot room, the glucose concentration increased slightly at 70 min after injury and then increased or remained constant (fig. 1A). In the control animals...
which were transferred to the chambers and back again there was a more rapid and continuing increase in glucose concentration (fig. 1c). In rats given halothane the initial increase was more rapid ($P < 0.005$ (group A); $P < 0.05$ (group C) by the Wilcoxon rank sum test), and more transient, as shown by a greater decrease in concentration later ($P < 0.02$, calculation of exact probabilities).

The experiment was repeated taking larger blood samples for the measurement of insulin, but omitting the second set of controls. The more rapid increase in plasma glucose in rats exposed to halothane was confirmed (six controls, seven halothane, $P < 0.001$, Wilcoxon rank sum test) despite the occurrence in the controls of increases in plasma glucose concentrations like those in figure 1c, presumably as a result of the extra blood loss. In both groups plasma insulin concentrations increased after the first sampling time ($P < 0.001$, Wilcoxon rank sum test). The increase was greater in rats under halothane anaesthesia but not significantly so.

**Glucose metabolism during steady-state anaesthesia**

During isotope experiments, begun about 2 h after injury (table I), mean plasma glucose concentrations

<table>
<thead>
<tr>
<th>Time after injury (min)</th>
<th>Controls</th>
<th>Halothane</th>
</tr>
</thead>
<tbody>
<tr>
<td>First exposure to halothane</td>
<td>—</td>
<td>70</td>
</tr>
<tr>
<td>Injection of label</td>
<td>120</td>
<td>130</td>
</tr>
<tr>
<td>Last sampling time</td>
<td>260</td>
<td>270</td>
</tr>
<tr>
<td>Rate coefficients and rates*</td>
<td>~155</td>
<td>~190</td>
</tr>
</tbody>
</table>

* These are only given exactly at certain specific times after injection of label. These times covered a range of about 15 min in each group. Mean values are given.

**Table II. Linear regressions of plasma glucose concentration, $C_p$, on time after injury during isotope experiments**

Conscious rats

$C_p = 13.31 \pm 0.37 = - (0.0423 \pm 0.0085) (t - 213)$

Rats under halothane

$C_p = 14.00 \pm 0.28 = - (0.0175 \pm 0.0051) (t - 199)$

The equations only hold over the period covered by the experiments, 2–4.5 h after injury. The equations are in the standard form:

$C_p - \bar{C}_p \pm SEM = (\text{slope} \pm \text{SEM}) (t - \bar{t})$

where "bar" represents a mean value, and $t$ is time after injury. Values of $C_p$ are mmol litre$^{-1}$, time in min.

**Figure 2. Rate coefficients with standard errors for glucose utilization in rats at 30 °C ambient estimated using [5-3H]-glucose.** The results for non-injured rats are taken from Heath, Frayn and Rose (1977a). In those without halothane the range of plasma glucose concentration was too small for a regression line to be calculated.

**Figure 3. Rates of glucose utilization in rats at 30 °C ambient estimated using [5-3H]-glucose.** Details are as in the legend to figure 2.
decreased slowly and almost linearly with time (table II). Therefore, since the rats were not in a steady metabolic state, rigorous calculation of rates and rate coefficients required the application of non-steady-state theory (Heath and Barton, 1973), which gives values that are exact only at certain specified times after injection (table I).

The dependence of rate coefficients and rates, determined with [5-\(^3\)H]-glucose, upon the plasma glucose concentrations in conscious and anaesthetized injured rats can be accounted for mostly by the slight mismatching of times shown in table I. Amongst the injured rats within each of the four groups (two labels, with and without halothane) the rate coefficient was negatively correlated with plasma glucose concentration (table II). Mean values of rates are shown in figures 2 and 3. The pattern of results with [U-\(^14\)C]-glucose was very similar. The different ranges of plasma glucose concentrations in conscious and anaesthetized injured rats can be accounted for mostly by the slight mismatching of times shown in table I. Amongst the injured rats within each of the four groups (two labels, with and without halothane) the rate coefficient was negatively correlated with plasma glucose concentration (\(P<0.001\), Student’s \(t\) test), that is, in any one rat in a group the lower its rate coefficient the higher its plasma concentration. Rates were, however, similar in different rats within a group—in only one group, anaesthetized rats given [5-\(^3\)H]-glucose, was there a significant correlation with plasma glucose concentration (positive, \(P<0.01\), Student’s \(t\) test). Mean values of rates are shown in table III.

The reduction in the rate of glucose utilization caused by halothane in injured rats was accompanied by an increase in insulin concentration. One experiment in which this was shown is in table IV. In two

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Utilization</th>
<th>Replacement</th>
<th>Ratio of utilization rates</th>
<th>Plasma glucose concentration (mmol litre(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injured</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Halothane</td>
<td>[5-(^3)H]-glucose</td>
<td>7.0 ± 0.1</td>
<td>6.6 ± 0.2</td>
<td>1.241 ± 0.007</td>
</tr>
<tr>
<td>Conscious</td>
<td>[5-(^3)H]-glucose</td>
<td>10.2 ± 0.2</td>
<td>9.2 ± 0.3</td>
<td>1.250 ± 0.007</td>
</tr>
<tr>
<td>Non-injured</td>
<td>[5-(^3)H]-glucose</td>
<td>6.4 ± 0.1</td>
<td>6.4 ± 0.1</td>
<td>1.171 ± 0.010</td>
</tr>
<tr>
<td>Conscious</td>
<td>[5-(^3)H]-glucose</td>
<td>7.7 ± 0.3</td>
<td>7.7 ± 0.3</td>
<td>1.131 ± 0.006</td>
</tr>
</tbody>
</table>

| Treatment | [U-\(^14\)C]-glucose | | | |
|-----------|---------------------|| | |

other experiments plasma insulin concentrations were compared in rats after 90 min exposure to halothane and in unanaesthetized rats at the corresponding time after injury. In both experiments the insulin concentrations in rats under halothane anaesthesia were all significantly more variable (F-test) than those in controls, the statistical significances of differences were calculated by the Wilcoxon rank sum test

<table>
<thead>
<tr>
<th></th>
<th>Halothane ((n = 11))</th>
<th>Controls ((n = 12))</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose (mmol litre(^{-1}))</td>
<td>13.8 ± 1.1</td>
<td>13.5 ± 0.6</td>
<td>n.s.</td>
</tr>
<tr>
<td>Liver glycogen (g kg(^{-1}))</td>
<td>14.9 ± 2.5</td>
<td>25.2 ± 1.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma insulin (mu. litre(^{-1}))</td>
<td>86.4 ± 11.2</td>
<td>60.9 ± 2.6</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

In injured rats halothane did not alter the ratio of the rates determined with the two labels (table III).
HALOTHANE AND GLUCOSE METABOLISM

This ratio is a measure of the fraction of the glucose carbon recycled. The fractional contribution of liver glycogenolysis to glucose production was therefore unaffected by halothane, and the absolute rate of glycogenolysis decreased in proportion to the reduction in glucose production (table III). However, the liver glycogen concentration was found to be lower at this stage in rats under halothane anaesthesia (table IV), showing that a rapid breakdown must have occurred at an early stage of anaesthesia.

DISCUSSION

The effects on glucose metabolism of the scald injury can be regarded as typical of the ebb phase after severe, non-haemorrhagic injury (Heath and Corney, 1973). They cannot be attributed to hypoxia, since tissue oxygenation was essentially normal except in the regions of damage (Threlfall, 1968; Barton, 1971).

The effects of halothane after injury were those expected from earlier results in uninjured rats (Biebuyck and Lund, 1974; Heath, Frayn and Rose, 1977a). During the induction of anaesthesia hyperglycaemia was increased (fig. 1b), presumably because of rapid glycogenolysis, accounting for the depletion of liver glycogen observed later (table IV). After 2 h of anaesthesia glucose utilization was reduced (fig. 3, table III) in spite of an increase in plasma insulin concentration. Therefore, halothane increased the already considerable insulin resistance present in scalded rats (Frayn, 1975, 1976b).

In man, halothane causes hyperglycaemia in the absence of premedication (Greene, 1968), but there is no evidence as to whether it increases the hyperglycaemia of injury. A transient increase such as that found in the rat might not be observed. Inhibition of glucose uptake by halothane has been observed in the dog (Galla, 1967). The only effect in the rat that was definitely at variance with findings in man was the increase in plasma insulin concentration, which in man is usually unaffected or reduced (Oyama and Takazawa, 1971; Yoshimura, Kodama and Yoshitaka, 1971). If insulin release is under tonic sympathetic inhibitory control in the rat as in man and other species (Werrbach et al., 1970; Robertson and Porte, 1973; Bloom and Edwards, 1975; Kaneto, Kajinuma and Kosaka, 1975) the difference may be a result of greater depression of sympathetic activity in the rat, for which there is some evidence from comparison of the results of Roizen and others (1974) on the rat and of Dobkin and others (1972) on man.

In view of the extent to which halothane exacerbated some of the effects of injury on glucose metabolism in the rat, it now seems highly desirable to see whether the same holds true in man.

ACKNOWLEDGEMENTS

We thank A. G. Watts and Miss P. F. Maycock for technical assistance.

REFERENCES


It has been studied the carbohydrate metabolism in two stages: after an injury non-lethal caused by scalding. The metabolism of glucose in rats following this have been studied: during and shortly after the induction and again 2 hours after the anesthesia.


--- --- --- --- (U-14C)-glucose, aumentó las concentraciones d'insuline en el plasma y disminuyó las concentraciones de glucogéne

WIRKUNG VON HALOTHAN AUF DEN GLUKOSESTOFFWECHSEL NACH VERLETZUNG IN DER RATTE

ZUSAMMENFASSUNG


LOS EFECTOS QUE EJERCER EL HALOTANO SOBRE EL METABOLISMO DE GLUCOSA EN LA RATA DESPUÉS DE RECIBIR ESTA UNA HERIDA

SUMARIO

Se investigaron los efectos que la anestesia de halotano ejerce sobre el metabolismo de glucosa en ratas después que éstas hayan recibido heridas no letales por escaldadura. Se indujo anestesia al cabo de 70 min de infusirse las heridas. Se estudió el metabolismo de la glucosa en dos etapas: durante y corto tiempo después de la inducción. Se hicieron comparaciones con ratas conscientes al cabo de un período semejante. Todas las ratas se encontraban en una temperatura ambiental de 30 °C, cercana a la termoneutralidad. Durante y corto tiempo después de la inducción de anestesia, el halotano provocó un rápido aumento en la concentración de glucosa en la plasma, que al cabo de 30 min había comenzado a volver a los valores de los animales de control heridos; así aumentó la producción como la utilización de glucosa. También aumentaron las concentraciones de insulina. Sin embargo, al cabo de 2 h de exposición, el halotano disminuyó la producción y utilización de glucosa, según se determinó con [5-3H]- y [U-14C]-glucosa, aumentando las concentraciones de insulina en la plasma y disminuyendo las concentraciones de glucocéno en el hígado, es decir, había exacerbado los efectos bien conocidos de heridas en la rata, incluyendo la resistencia a la insulina. La hiperglicemia no fue aumentada.

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