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INSULIN SECRETION AFTER INJURIES OF DIFFERING SEVERITY IN THE RAT

K. N. FRAYN

From the Experimental Pathology of Trauma Section, MRC Toxicology Unit, Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey SM5 4EF

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Summary.—The effects on insulin secretion of injuries of differing severity have been studied in the rat. The injuries used were dorsal scalds to 20% and 40% of the body surface area, and a 4-h period of bilateral hind-limb ischaemia. These injuries resulted in 48 h mortality rates of 0/10, 7/10 and 5/10 respectively. Rats were studied 1·5–2 h after scalding or removal of tourniquets. The blood glucose concentration was markedly raised after all these injuries, and the plasma insulin concentration was also raised, so that the insulin to glucose ratio in any group did not differ significantly from that in non-injured controls. Injection of glucose (0·5 g/kg i.v.) induced a rise in insulin concentration in all groups, although the insulin to glucose ratio after the lethal 40% scald was lower than in control rats. It was concluded that in the rat normal insulin secretion is maintained even after lethal injuries, although some suppression of the insulin response to exogenous glucose may occur. Insulin resistance is more important in the rat than impairment of insulin secretion even at an early stage after injury.

HYPERGLYCAEMIA and impairment of glucose utilization are well-known consequences of injury in man and other animals. The work of Allison and others (Allison *et al.*, 1967, 1968) showed that in man there may be suppression of glucose-stimulated insulin secretion in shock, and this finding has been confirmed in many species including the rat, in which the insulin response to i.v. glucose administration is abolished after Noble–Collip drum trauma (Vigaš *et al.*, 1973). However, it seemed unlikely from studies of glucose utilization in the rat that suppression of insulin release could provide a complete explanation for the impairment of utilization which follows either scald or ischaemic injury (Heath and Corney, 1973). This was confirmed by the demonstration (Frayn, 1975) that after a 20% scald injury insulin secretion and its response to glucose administration were substantially normal, implying a marked degree of insulin resistance at an early stage after injury. This lack of impairment of insulin secretion was surprising, and raised the question as to whether this might be related to lack of severity of the injury, or perhaps to some peculiarity of this type of scald injury such as the use of anaesthesia during its production. In view of the therapeutic importance of understanding the basis for the impairment of glucose utilization following injury these studies have now been extended both to a more severe burn injury, and to bilateral hind-limb ischaemia, a lethal injury in which the animals are not under general anaesthesia at the stage of production of fluid loss.

MATERIALS AND METHODS

Animal methods

Rats.—These were male Porton-Wistar albinos, fed on MRC diet 41B and kept from weaning at 20° ambient with 12 h light per day from 07.00 hours. Food was removed and rats weighed at 09.00 hours on the day of experi-

ment. Rats to be injured weighed 231–252 g (mean 242 g), and others 217–260 g (mean 247 g).

Scalding.—Scalds to 20% or 40% of the body surface area were produced by a modification of the method of Bailey *et al.* (1962). Rats were anaesthetized with ether and the dorsal fur clipped to expose the required area. They were then strapped to the underside of a flat metal plate (9·0 cm across) with a string on each limb and round the tail, and transversely across the middle of the rat. Respiration was not obstructed. A sling was used to hold the head and ears clear of the water. This arrangement was found to expose a large area of the dorsum for scalding without risk of water reaching the abdomen. The complete frame was then lowered to a predetermined height above the water surface, using a rotating arm with screw adjustment (Bailey *et al.*, 1962). Scalding was performed using water at 83° with 30 s immersion; these conditions produce a full skin-thickness burn with minimal additional damage (Stoner, H. B. and Little, R. A., unpublished work; Ófeigsson *et al.*, 1972). Post-mortem examination of rats dying from a 40% scald showed hyperaemia of deep fascia over the dorsal muscle, extending round to the flank, with occasional hyperaemia in superficial muscle layers to a depth of about 1 mm. There was no evidence visible to the naked eye either of heat coagulation of muscle or of damage to abdominal viscera.

The apparatus was calibrated in terms of height of the metal plate above the water surface rather than by measuring displacement of water (Bailey *et al.*, 1962), which was found to be unreliable due to variable surface-tension effects. Calibration was effected by dipping anaesthetized rats into a dye solution, killing the rat after removal, clipping all the fur and immediately removing the skin. The dyed area was removed and weighed, and expressed as a percentage of the weight of the whole skin. Arbitrarily the skin covering the extremities of the limbs, the head from the ears forward, and the tail, was not included. Provided that one operator always performed the setting up, and that rats of a narrow weight range (240–250 g) were used, this method produced reliable results. The estimated standard error ranges for predicted scalds of 20 and 40% were 18·7–21.3% and 38.9–41·1% respectively.

Scalding was performed between 11.00 and 13.00 hours. The ether anaesthesia lasted 4–5 min.

Bilateral hind-limb ischaemia.—This was produced by application of tourniquets (Rosenthal, 1943) under ether anaesthesia (lasting about 3 min). The tourniquets were removed without general anaesthesia after 4 h, which was at 13.00–14.30 hours.

Controls.—Control (non-injured) rats were given a similar short period of ether anaesthesia and then left together in communal cages. Their fur was not clipped. The effects of individual housing and clipping of fur were assessed in additional rats by clipping the fur under ether anaesthesia as for a 40% scald, and leaving the rats in individual cages.

Glucose injection.—Rats to be injected with glucose had a tail-vein cannula inserted at the time of scalding, tourniquet application, or control anaesthesia, and were kept in restraining cages.

Experimental plan.—Some rats were killed by decapitation 90–120 min after injury and blood collected in a heparinized beaker. Colon temperatures were measured just before decapitation. In others glucose (0·5 g/kg as 50% w/v solution) was injected *via* the tail-vein cannula 2·5–3 min before killing. Other rats were provided with food and water and left for determination of 48 h mortality; their colon temperatures were measured at about 17.00–18.00 hours on the day of experiment, and in survivors at 09.00 hours the following day.

Analytical methods

Whole blood glucose concentration was measured using hexokinase (Schmidt, 1961) after deproteinization (Somogyi, 1930). Plasma insulin was measured using a double antibody radioimmunoassay based on that of Morgan and Lazarow (1963) with rat insulin (Novo Research Institute, Denmark) as standard. Microhaematocrit values were corrected for entrained plasma (Heath, 1973).

Statistical methods

Statistical methods were based on those of Snedecor and Cochran (1967). Means were compared using either the *t* test, modified when necessary for samples of unequal variance, or the Mann–Whitney non-parametric test.

RESULTS

General effects of injury

After all three types of injury colon temperatures were significantly reduced and haematocrits raised during the following few hours (Table I). After 20% scald and hind-limb ischaemia the fall in colon temperature was similar to that observed by Stoner (1958, 1968). The depression of colon temperature was significantly greater after a 40% than after a 20% scald, but less marked at this stage after hind-limb ischaemia. In clipped controls (housed individually) the colon tempera-

TABLE I.—*Colon Temperatures, Haematocrit Values and Mortality Rates in Injured Rats*

Treatment	Haematocrit (%)	Colon temperature (°C) 1·5–2 h	3–6 h	19–22 h	48 h Mortality (No. of deaths out of 10)
Controls	41·8±0·7 (6)	38·1±0·2 (8)	37·7±0·1 (6)	38·4±0·2 (6)	0
Clipped, non-injured	—	37·4±0·1 (6)*	37·8±0·1 (6)	37·4±0·1 (6)*	0
20% scald	55·4±0·6 (9)**	33·9±0·3 (8)**	34·2±0·3 (10)**	38·2±0·2 (10)	0
40% scald	56·1±0·9 (9)**	31·6±0·3 (9)**	30·3±0·4 (10)**	34·2±2·2 (4)	7
Ischaemia	56·3±0·6 (7)**	35·5±0·4 (9)**	35·4±0·2 (10)**	35·9±0·5 (5)*	5

Scald or bilateral hind-limb ischaemia were produced as described in the text. In some rats haematocrits and colon temperature were measured 1·5–2 h after injury or control anaesthesia. In others colon temperatures were measured at about 17.00 h on the day of injury (3–6 h later) and in survivors at 09.00 h the following day (19–22 h later). Results are means ± S.E.; numbers of rats are shown in parentheses. Asterisks indicate significant difference from controls; $*P < 0.01$; $**P < 0.001$; in addition colon temperatures at 1·5–2 h and 3–6 h after injury were significantly lower ($P < 0.001$) after 40% scald than after 20% scald.

ture was slightly reduced compared with unclipped controls (housed together). All three injured groups were indistinguishable on the basis of their haematocrits.

All rats dying from these injuries did so during the following night (survival time < 21 h) except for one death 24 h after 40% scald. The 5/10 mortality rate after hind-limb ischaemia is significantly lower ($P < 0.01$; χ^2 test) than the 85% sometimes found in these laboratories (Stoner, 1961), and this must represent either a difference in susceptibility of the animals or else a slight difference in technique. No deaths were found after 20% scald, as is always the case in our laboratories (Stoner, 1968). By the following morning surviving rats were generally recovering as shown by their colon temperatures.

Blood glucose and plasma insulin concentrations (Table II)

In all groups of injured rats both the blood glucose and the plasma insulin concentrations were raised after 1·5–2 h compared with controls. The blood glucose concentration was significantly higher after 40% scald than after 20%, and intermediate after hind-limb ischaemia. The plasma insulin to blood glucose ratio tended to be raised after injury; this effect was not significant in any single group, but was significant ($P < 0.05$; Mann–Whitney test) comparing all injured rats (I/G, mean ± S.E.; 4·5 ± 0·8 μU/μmol; n = 26) with controls.

After injection of glucose, there was a significant increase in plasma insulin concentration in each group ($P < 0.05$). The plasma insulin concentrations were

TABLE II.—*Blood Glucose and Plasma Insulin Concentrations after Injury in the Rat*

Treatment	Without glucose n	Glucose mmol/l	Insulin μU/ml	I/G μU/μmol	After glucose n	Glucose mmol/l	Insulin μU/ml	I/G μU/μmol
Controls	13	6·5±0·1	16± 4	2·4±0·6	11	13·1±0·2	135±11	10·4±0·9
20% scald	8	10·6±0·6***	55±14*	6·0±2·2	5	19·4±1·7*	135±27	7·4±1·8
40% scald	9	12·7±0·7***	57±11**	4·6±0·9	5	19·8±0·8**	129±18	6·5±0·8**
Ischaemia	9	12·1±0·8***	34± 5*	3·0±0·6	9	16·2±0·7**	129±15	8·4±1·4

Concentrations were measured 1·5–2 h after injury or control anaesthesia. The ratio of plasma insulin to blood glucose concentrations (I/G) was calculated for each rat. Glucose (0·5 g/kg i.v.) was injected 2·5–3 min before killing. Results are means ± S.E. Asterisks show significant difference from controls; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. In addition the blood glucose concentration (without glucose injection) was significantly higher ($P < 0.05$) after 40% than after 20% scald.

almost identical after glucose injection in all groups. Since the blood glucose concentrations were higher in the injured rats, however, the insulin to glucose ratio tended to be lower than in the controls. This reduction was significant ($P < 0{\cdot}01$) after the 40% scald.

DISCUSSION

The previous finding (Frayn, 1975) that insulin secretion in the rat was unaffected by a 20% scald injury was interesting firstly because suppression of insulin secretion seemed to be a general response in many species to various types of trauma (see Frayn, 1975, for references) and secondly because it showed the occurrence of insulin resistance at an early stage after injury. However, this 20% scald injury is not acutely lethal, and the present studies were performed in order to extend these observations to the effects of more severe injuries.

The 40% scald used in the present work was considerably more severe than the 20% as judged by its 48-h mortality rate, by the hyperglycaemia produced, and by the depression of colon temperature. It is probably the largest burn injury which could be produced in the rat without damage to internal organs. The ischaemic injury studied was also more severe than a 20% scald in terms of mortality, and differs in that the fluid-loss-producing stage (removal of the tourniquets) is not carried out under general anaesthesia. At the time at which these studies were carried out, however, it was probably the least severe with respect to fluid loss; although the haematocrits were raised equally after all three injuries, there is some red cell loss after scalding (Heath, 1973) but not after hind-limb ischaemia (Little, R. A. and Elebute, E. A., unpublished work), showing that the loss of plasma volume after scalding must have been correspondingly greater.

Despite the severity of all these injuries insulin secretion, in the absence of stimulation with exogenous glucose, was maintained in the injured rats. The insulin to glucose ratio was in fact elevated nearly two-fold comparing all injured rats with controls, suggesting that insulin resistance was present but that pancreatic insulin output could still be increased as a compensatory response. All groups responded to injection of glucose with a marked rise in plasma insulin concentration, although this response was significantly suppressed after the more severe scald injury.

Suppression of insulin secretion in shock is probably entirely attributable to increased catecholamine release, since it does not occur after adrenal medullectomy (Vigaš *et al.*, 1973), and yet considerable adrenaline release is known to occur after hind-limb ischaemia (Stoner and Westerholm, 1969). These results therefore show that suppression of basal insulin secretion is not a universal consequence of severe or even of lethal injury, irrespective of the use of an anaesthetic agent and irrespective of known catecholamine release. The results are in contrast with the complete abolition of glucose-stimulation of insulin secretion found after Noble–Collip drum trauma (Vigaš *et al.*, 1973) and emphasize the peculiar and highly stressful nature of that form of injury.

An important conclusion emerges from these findings. The fall in colon temperature after these injuries is due to decreased heat production, total heat loss at this stage after scald (Miksche and Caldwell, 1968) or ischaemic (Stoner and Pullar, 1963) injury in the rat being less than in controls. Since in the normal post-absorptive rat at 20° glucose utilization accounts for about 40% of the O_2 consumption (Heath and Corney, 1973), this fall in heat production probably involves a drop, or at least no increase, in the rate of glucose utilization, as has been shown after both 20% scald and hind-limb ischaemia (Heath and Corney, 1973). For this to occur despite marked elevation of both blood glucose and plasma insulin levels implies a considerable loss of sensitivity to insulin, thus confirming the

conclusion drawn from studies after 20% scald (Frayn, 1975) that insulin resistance may be an important and early response to injury.

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