Effects of Insulin Hypoglycaemia in the Sheep on Jugular Haematocrit and Plasma Corticosteroid Concentrations

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Abstract

Insulin hypoglycaemia, by acting as a stressor, caused an increase in plasma corticosteroid concentration in sheep. It did not increase jugular haematocrit in splenectomized sheep, but caused an increase, presumably by splenic contraction, in the following sheep: two control, one with one adrenal cortex as its only adrenal tissue, two with denervated spleens, and two splanchnicotomized animals. These preparations showed that insulin hypoglycaemia can cause a splenic contraction in the absence of an increase in plasma adrenaline and after splenic extrinsic denervation.

Introduction

Cortisol and insulin affect plasma glucose concentration in sheep in opposing ways: increased levels of cortisol elevate the plasma glucose and this stimulates increased secretion of insulin which tends to lower it (Bassett and Wallace 1967). Insulin injections therefore should increase the secretion of corticosteroids from the adrenal cortex in sheep as it does in man (Bliss et al. 1954; Christy et al. 1957) but we have not found any reports which show this.

Insulin-induced hypoglycaemia also stimulates the adrenal medulla to increase its output of adrenaline (Setchell and Waites 1962) and one of the consequences reflected in the circulatory system of the sheep would be an elevated jugular haematocrit, since injected adrenaline contracts the spleen which expels its packed red cells into the circulation, raising the jugular haematocrit (Turner and Hodgetts 1959; Dooley et al. 1972).

This paper reports the effects of insulin hypoglycaemia on the concentration of corticosteroids in jugular plasma and on the jugular haematocrit in sheep. Insulin hypoglycaemia was found to be a powerful stimulant of adrenal corticosteroid secretion, and it also led to the contraction of the spleen. The agent contracting the spleen could not be ascertained.

Materials and Methods

Sheep

Mature Merino wethers, housed in metabolism cages and given 800 g lucerne chaff daily at about 0810 h were used. The surgical preparation of the animals and the techniques used are described below.
Surgery

(i) Anaesthesia

Anaesthesia, induced with pentobarbitone sodium, was maintained after intubation with an ether–oxygen mixture.

(ii) Splenic preparations

The left last rib was removed and the peritoneal cavity entered. The attachments of the spleen to the rumen and diaphragm were broken down by blunt dissection and the pancreas was dissected free from the splenic blood vessels. Then the sheep were either splenectomized, or their spleens were denervated (described below) and the laparotomy was closed.

Splenectomy. The splenic pedicle, comprising the artery, vein and accompanying tissue (lymphatics, nerves and connective tissue), was divided between ligatures and the spleen was removed.

Denervation of the spleen. The splenic artery and vein were separated from the tissues of the splenic pedicle. These tissues were then divided into two by the removal of about 1 cm of tissue.

(iii) Adrenal gland preparation

An incision was made behind the left last rib for access to the left adrenal gland. Access to the right adrenal gland was provided by a separate incision on the right side and transection of the right last rib.

Right adrenalectomy, and left adrenal denervation and demedullectomy were performed on one sheep as follows. The left adrenal gland was denervated by severing all nerve fibres passing to it from the coeliac plexus. Incisions were then made into its cortex and the medullary tissue was scraped out (Sakai 1967). One week later the right adrenal gland was extirpated (Goding and Denton 1957). This animal is described as a ‘unilateral cortical’ sheep.

(iv) Splanchnicotomiy

The general approach was that described for adrenal gland preparations. The right and left coeliac plexuses were identified and denervated in separate operations by severing the splanchnic nerve at the level of the diaphragm together with other fibres passing to the coeliac plexus from the spinal cord.

Post-operative Treatment

All animals recovered uneventfully and no hormone therapy was necessary. Sheep were not used until at least 4 weeks after surgery, when they were placed in metabolism cages and were trained to accept handling in the neck region without showing alarm.

Blood Sampling

A jugular vein of each sheep was cannulated aseptically at least 16 h before an experiment. The sheep were also weighed at this time.

Each blood sample of 2·5 ml was withdrawn into a dry 5-ml syringe. 0·5 ml of blood was added to stoppered vials containing 10 i.u. dried heparin (‘Pularin’, Evans Medical Pty Ltd) and was used for estimating haematocrit.

The remainder was added to a vial containing 40 i.u. dried heparin plus 4 mg potassium fluoride. After gentle mixing the sample was placed in a bath of iced water for up to 20 min. The sample was then centrifuged at room temperature for 5 min at 3000 g and the plasma was removed. Some plasma was then frozen to −20°C pending corticosteroid analyses; the remainder was held for 6 h at room temperature in stoppered vials before glucose concentration was measured. The delay caused no change in the glucose concentration.

Estimating the Haematocrit

The micro method of Dooley et al. (1974) was used. Blood collected into dried heparin (20 i.u./ml blood) was centrifuged at a relative centrifugal force of 10,000 g for 8 min within 1 h of collection. Microhaematocrit could be measured this way in replicate samples with S.D. of 0·002 over the haematocrit range of 0·12–0·85.
Insulin Hypoglycaemia Effects in the Sheep

Determination of Plasma Glucose Concentration

The alkaline potassium ferricyanide method of Hoffman (1937) as adapted to the Auto Analyzer was used. The mean glucose concentration (± s.d.) for 12 replicates was 76·9±1·0 mg/100 ml, and the recovery of glucose from aqueous solutions and from plasma was 98·9±1·9%.

Measurement of Plasma Corticosteroid Concentration

The competitive protein-binding method of Bassett and Hinks (1969) was used to measure total corticosteroids (i.e., those bound to plasma protein plus free corticosteroids) in the plasma; the binding of corticosteroid to protein is weak and is disrupted by protein precipitation (Daughaday 1959). Of the steroid assayed in sheep plasma, 90% is cortisol and 10% is corticosterone. No cortisone is measured by this method. The reproducibility of the method was investigated at regular intervals. In a typical experiment, the mean concentration of plasma corticosteroids (± s.d.) for 10 replicates was 3·1±0·5 ng/ml.

The recovery of added cortisol to a sample of plasma of known corticosteroid concentration was 68, 66 and 61% at added cortisol concentrations of 24, 13·2 and 8 ng/ml respectively.

Insulin Injections

The dose of insulin (B.P. Wellcome), nominally 1 i.u./kg body weight, was diluted to about 4 ml with sterile NaCl solution (0·9%, w/v). The amount injected was estimated from a weight difference.

Experimental

Food was withheld during experiments. Blood sampling started at c. 0700 h and continued for 3 h. Insulin was then injected through the jugular cannula and further samples were taken.

Sheep showing symptoms of stage III hypoglycaemia (Reid 1951) were given about 15 g glucose as a sterile solution via the jugular cannula. The experiment was ended and the sheep were fed.

Results

Plasma Corticosteroid Concentrations

The intravenous injection of insulin increased plasma corticosteroid concentrations by between 5- and 11-fold in 60 min in control, unilateral cortical, and splanchnicotomized sheep (see Fig. 1).

Plasma Glucose Concentration

Plasma glucose concentration was stable in all sheep before injection, with means ranging between 55 and 65 mg/100 ml, except in sheep 572 (splenectomized; Fig. 3b) which had a mean of 42 mg/100 ml.

Plasma glucose concentration decreased in the 2 h following insulin injections by 60–70% to values between 16 and 24 mg/100 ml in sheep with innervated adrenal glands (Figs 2 and 3). Larger decreases (78–83%) to values of 9–13 mg/100 ml occurred in sheep with denervated adrenal glands (Fig. 4). Minima were not determined in the first experiment on the unilateral cortical sheep (Fig. 4b, insulin dose 1·11 i.u./kg body weight) or in the splanchnicotomized sheep (Fig. 4d): experiments on them were terminated within 90 min of injection when stage III hypoglycaemic symptoms appeared. When the experiment on the unilateral cortical sheep was repeated on another occasion (Fig. 4b, insulin dose 0·92 i.u./kg body weight), stage III hypoglycaemic symptoms were again evident. This time, glucose solution was not injected into this animal, and it died 36 h later. The maximum reduction in plasma glucose concentration, however, was within the range given above.
**Jugular Haematocrit**

The haematocrit commonly decreased slightly in all except splenectomized sheep in the period preceding the injection of insulin. Pre-injection values for all sheep were in the range of 0.23–0.32.

Injected insulin rapidly increased the haematocrit in the two intact sheep by 28% and 37% within 60–90 min (Fig. 2). There was no change in haematocrit in the splenectomized sheep (Fig. 3a). Haematocrit increased by about 22% in sheep with denervated spleens (Fig. 3c) and by 31% in the unilateral cortical sheep (Fig. 4a, insulin dose 1.11 i.u./kg body weight). On a second occasion, insulin injected into the latter sheep increased the haematocrit by a maximum of 43% in 110 min, 20 min after the onset of stage III hypoglycaemia (Fig. 4a, insulin dose 0.92 i.u./kg body weight).

Severe hypoglycaemic symptoms limited experiments on splanchnicotomized sheep to 60–90 min after injection. Haematocrit did not respond immediately to insulin, and after 30 min it was slightly less than the pre-injection value. Haematocrit then increased by 17% and 9% in the two sheep in the remaining time (Fig. 4c).

Haematocrit remained elevated for several hours in intact sheep, in sheep with denervated spleens and in the terminal experiment on the unilateral cortical sheep.

**Discussion**

The data show that insulin hypoglycaemia results in a dramatic increase in plasma corticosteroid concentration in sheep as it does in man (Bliss *et al.* 1954; Christy *et al.*
1957). The response is presumably due to the stimulation of ACTH secretion through the action of hypoglycaemia on the hypothalamus. Insulin hypoglycaemia is apparently a potent stressor.

Insulin injections reduced the plasma glucose concentration in all sheep. In intact and splenectomised sheep, and in sheep with denervated spleens, the concentration fell by about 65% and only mild hypoglycaemic symptoms (stage I, Reid 1951) were observed. These data suggest that the spleen of the sheep is not involved in any essential manner with the glucose metabolism of the animal. In contrast, glucose concentration fell by about 80% in sheep with denervated adrenal glands; the accompanying severe hypoglycaemic symptoms were counteracted by intravenous injections of glucose. Other workers have found similar results (Jarrett and Potter 1953; Findlay and Robertshaw 1965). Adrenaline increases plasma glucose concentration but denervating the adrenal glands prevents its release during insulin hypoglycaemia (Setchell...
and Waites 1962). These mechanisms account for the greater reduction in plasma glucose and for the more severe symptoms in the unilateral cortical and splanchnicotomized sheep than in the other sheep.

Haematocrit in intact sheep increased rapidly after insulin injections and maximum increases of about 30% were reached after 60 min (Fig. 2). The haematocrit increase was entirely due to a contraction of the splenic smooth muscle and the ensuing expulsion of red cells, since haematocrit failed to increase in splenectomized sheep after insulin had been injected into them (Fig. 3a).

This contraction would appear to be mediated by a hormone; cutting the splenic nerves, which effectively denervates the spleen of the sheep (von Euler and Purkhold 1952) did not abolish the haematocrit response (Fig. 3c).

Splanchnicotomy, which denervates the spleen and adrenal glands and reduces the output of catecholamines from them, failed to abolish the haematocrit increase. The onset of the response, however, was delayed by about 30 min (Fig. 4c) possibly as a consequence of the extensive alterations of cardiovascular function brought about by splanchnicotomy (Edwards 1971; Edwards and Silver 1972).

Further evidence excluding catecholamines from the adrenal medulla as possible mediators of splenic contraction is provided by the increase in haematocrit in a sheep whose total adrenal tissue consisted of only one denervated cortex (unilateral cortical sheep; Fig. 4a).

It is interesting to speculate on the identity of the mediator. A possible contender—a hormone from the corticosteroid group—can be excluded on the grounds that no direct experimental evidence has been found in the sheep to suggest a direct effect on splenic smooth muscle of corticosteroids. Glucagon, which exerts widespread effects on many splanchnic organs has little effect, however, upon splenic vascular smooth muscle (Kock et al. 1970). Available evidence suggests gastrin as a possible
mediator; insulin hypoglycaemia in the sheep stimulates the release of this hormone into the blood (Hill 1960). Gastrin is able to release acetylcholine from storage sites in the digestive tract (Vizi et al. 1973), and acetylcholine is also found within the spleen and is part of a mechanism that releases noradrenaline from nerve endings (Brandon and Rand 1961) where the released noradrenaline in turn contacts the splenic smooth muscle. It is possible, therefore, that gastrin released from tissue stores during insulin hypoglycaemia may be the splenic contractor.

Finally, attention is drawn to the ability of the denervated sheep spleen to store and release packed red cells (Fig. 3c). This is contrary to the finding of Barcroft and Poole (1927) that denervation prevented the operation of the red cell concentrating mechanism in the dog.

References

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