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ENDOCRINE RESPONSES TO INSULIN HYPOGLYCAEMIA IN THE YOUNG CALF

BY S. R. BLOOM,* A. V. EDWARDS, R. N. HARDY, KRYSTINA W. MALINOWSKA AND MARIAN SILVER

From the Physiological Laboratory, University of Cambridge, Cambridge CB2 3EG and the Institute of Clinical Research, the Middlesex Hospital, London, W.1

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SUMMARY

1. Variations in the output of glucocorticoids and catecholamines from the right adrenal gland, in response to insulin hypoglycaemia, have been investigated in calves 2–5 weeks after birth. These have been correlated with changes in the concentration of glucocorticoids and glucagon in arterial plasma.

2. Moderate hypoglycaemia for a limited period (0·1 u. insulin/kg), elicited a prompt increase in steroid output from the adrenal gland followed by a significant rise in plasma glucagon concentration. By comparison, changes in both catecholamine output and peripheral plasma glucocorticoid concentrations were found to be trivial in this group of animals.

3. Administration of a larger dose of insulin (0·5 u./kg) produced a more substantial fall in plasma glucose concentration followed by spontaneous recovery within 2–3 hr. This stimulus elicited the release of greater amounts of both cortisol and corticosterone, followed by a significant increase both in the output of adrenaline and in plasma glucagon concentration. Increase in steroid output was accompanied by an increase in adrenal blood flow and was associated with elevated concentrations of both steroids in arterial plasma.

4. The adrenal cortical response and associated changes in plasma steroid concentration were found to be transient even in response to persistent and intense hypoglycaemia (4 u. insulin/kg). The increase in plasma glucagon concentration in this group of animals was not significantly greater than that produced by smaller doses of insulin. However, substantial amounts of adrenaline (78 ± 14 ng. kg^{-1} min^{-1}; maximum; $n = 9$) together with a little noradrenaline (10 ± 3 ng. kg^{-1} min^{-1}; maximum; $n = 9$) were released from the right adrenal gland under these conditions.

* Present address: Department of Medicine, Royal Post-graduate School of Medicine, Hammersmith Hospital, London, W.12.

5. Changes in adrenal blood flow could be related to adrenal glucocorticoid output in calves given 0·1 or 0·5 u. insulin/kg. In animals given the largest dose of insulin adrenal blood flow was found to increase coincidentally with rising steroid output but this hyperaemia then persisted after steroid output had subsided to values within the normal range.

6. Calves given the largest dose of insulin (4·0 u./kg) invariably collapsed and convulsed after 2–3 hr, but these symptoms could not be related to any particular endocrine response. No clinical signs of hypoglycaemia were observed in the other animals.

7. The results are discussed in relation to previous studies of adrenal function in this and other species.

INTRODUCTION

Previous studies of the mechanisms which maintain blood glucose concentration in the young calf have established the importance of both the pituitary–adrenal axis and the sympathetic nervous system, even though different experimental techniques have sometimes yielded apparently conflicting results. Thus, bilateral adrenalectomy causes death from hypoglycaemia in these animals within 24–48 hr unless exogenous cortisol is administered (Comline & Edwards, 1965), whereas administration of large amounts of exogenous cortisol to normal calves produces no significant change in plasma glucose concentration at rest, or in the response to insulin (A. V. Edwards, unpublished observations). Substantial amounts of pancreatic glucagon are released in response to splanchnic nerve stimulation at low frequency in anaesthetized animals (Bloom, Edwards & Vaughan, 1973) but the tolerance to moderate hypoglycaemia of calves with cut splanchnic nerves does not differ from that of normal animals (Bloom, Edwards & Vaughan, 1974). Furthermore, the remarkable resistance of the new-born calf to severe hypoglycaemia has been shown to depend on the release of adrenaline, but not noradrenaline, from the adrenal medullae (Comline & Edwards, 1968), yet splanchnic nerve stimulation in anaesthetized calves at the same age produces a secretion of proportionately greater amounts of noradrenaline (Silver, 1960; Comline & Silver, 1966).

In the present study, the 'adrenal clamp' technique (Edwards, Hardy & Malinowska, 1974) has been employed to define the changes in the output of glucocorticoids and catecholamines from the right adrenal gland which occur during insulin hypoglycaemia in conscious calves. These changes have been related to the variations in glucocorticoid and glucagon concentration in the peripheral arterial plasma with the intention of establishing a more comprehensive picture of the hormonal responses to hypoglycaemia. The results show that, whereas the adrenal cortex is

extremely sensitive to this stimulus initially, glucocorticoid output is not maintained during prolonged hypoglycaemia. In contrast, the adrenal medulla responds relatively slowly when plasma glucose concentration falls but persistent hypoglycaemia elicits the release of large amounts of adrenaline and comparatively little noradrenaline.

Some of these observations have been published previously in a preliminary form (Hardy, Silver, Addison, Malinowska & Edwards, 1974).

METHODS

Animals

Pedigree Jersey calves were obtained from local farms shortly after birth and used at ages ranging from 17 to 38 days (27·7–41·5 kg body weight). The animals were kept in individual pens in the laboratory animal house and maintained on a diet of milk (6–8 pints/day). Food was withheld for at least 6 hr before surgery and for at least 14 hr before testing with insulin. Daily records were kept of the weight and rectal temperature of each animal and care was taken to avoid the use of animals that were not completely healthy. Animals in which either the output of glucocorticoids or catecholamines from the right adrenal gland was found to be elevated above the normal resting range at the time that insulin was given have been excluded from the series.

Experimental procedures

Anaesthetic, surgical, post-mortem and experimental control procedures were identical with those described in detail previously (Edwards, Hardy & Malinowska, 1974, 1975). Preparatory surgery involved removal of the right kidney and implantation of a specially designed clamp to permit collection of the whole of the effluent blood from the right adrenal gland periodically when required. A narrow-bore polyethylene catheter was inserted into either the right or left saphenous artery so that the tip lay in the abdominal aorta. This catheter was used subsequently to monitor aortic blood pressure and for collection of arterial blood samples. Recovery was invariably rapid and each of these animals consumed 3 pints milk within 30 min after anaesthesia was discontinued. Penicillin (600,000 i.u.) and dihydrostreptomycin (500,000 i.u.) (Distavone; Dista Products Ltd) were administered routinely prior to surgery.

Experiments were carried out the day after surgery, and at the same time of day, in order to avoid variations attributable to diurnal rhythms. In each case, samples of adrenal effluent and of arterial blood were collected at 30 min intervals for 2–3 hr before insulin was administered, in order to accustom the animals to the sampling procedure. Heart rate and aortic blood pressure were monitored continuously, by means of a Devices L221 pressure transducer connected to a Devices M19 or M2 recorder, and rectal temperature was recorded at intervals throughout the day.

'Six times recrystallized' bovine insulin (Boots Pure Drug Co. Ltd), was dissolved in acidulated sterile saline and injected intravenously at a dose of 0·1, 0·5 or 4·0 u./kg body wt. The animals were observed continuously throughout the course of each experiment and any clinical signs of hypoglycaemia noted. Violent convulsions invariably occurred between 120 and 180 min in animals given the largest dose of insulin. No signs attributable to hypoglycaemia were observed in the other animals. Convulsions were controlled by administration of a small amount of sodium pentobarbitone (May & Baker) (6·0 mg/kg body wt.). Careful scrutiny of the results has failed to reveal any change in the hormonal responses which could be ascribed to the

barbiturate. Furthermore, each of these animals recovered within minutes when exogenous glucose was injected intravenously at the conclusion of the experiment. Samples of arterial and of right adrenal effluent blood were collected at intervals before and after administration of insulin. Adrenal blood flow was estimated gravimetrically and corrected for haematocrit % before the output of steroids and catecholamines from the gland was computed.

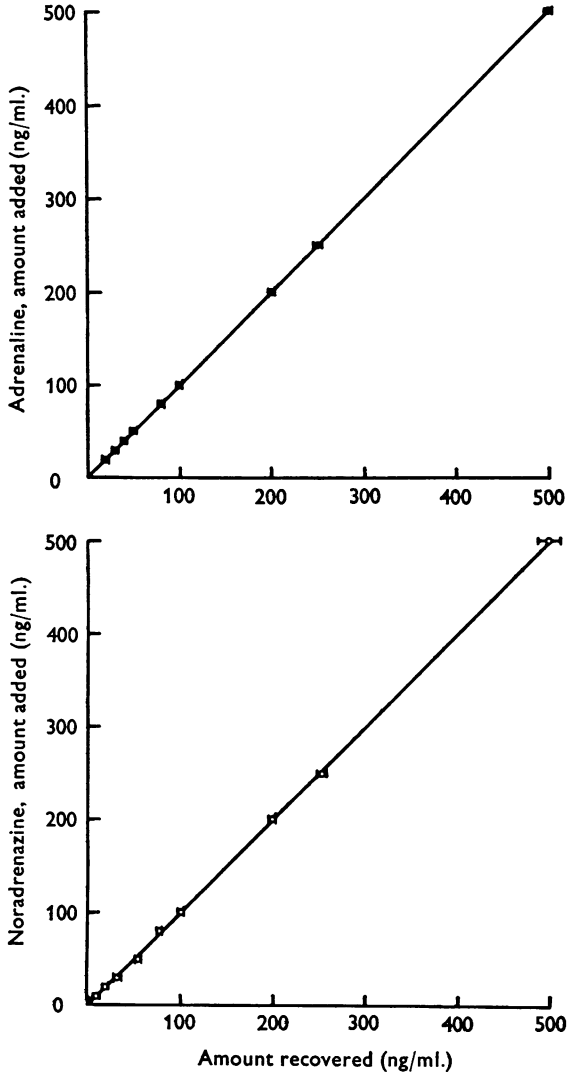

Fig. 1. Recovery of adrenaline and noradrenaline added separately to calf plasma in amounts ranging between 5 and 500 ng/ml. Horizontal bars: S.E. of each mean value (n = 5). Continuous line represents theoretical value assuming 100 % recovery.

Analytical procedures

Arterial and adrenal venous blood samples were collected into heparinized tubes containing a little EDTA and centrifuged immediately at +4° C. Plasma was stored at −20° C before analysis. Plasma glucose was estimated with glucose oxidase by means of a Beckman Glucose Analyser. Plasma glucagon was measured by radio-immunoassay using a highly specific antibody which showed no cross-reaction with enteroglucagon (Bloom *et al.* 1973). Cortisol and corticosterone were measured by competitive protein binding after preliminary separation on Sephadex LH 20 (Malinowska, Hardy & Nathanielsz, 1972).

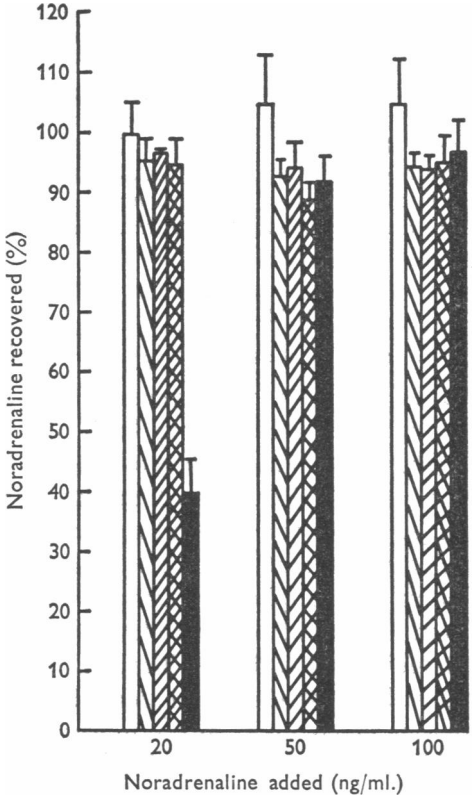

Fig. 2. % Recovery of noradrenaline (N) added to calf plasma in the presence of varying amounts of adrenaline (A). □ = no adrenaline present. ▨ A:N ratio 1:1. ▨ A:N ratio 2:1. ▨ A:N ratio 5:1. ■ A:N ratio 10:1. Vertical bars: S.E. of each mean value (n = 5).

Catecholamines were estimated by the trihydroxyindole method of Euler & Floding (1955). Differentiation between adrenaline and noradrenaline was obtained by measurement of fluorescence at two wave-lengths, 400/487 and 400/525 (activation/fluorescent) using a Perkin Elmer spectro-fluorimeter. A simplified extraction

procedure was adopted to allow large numbers of samples to be processed quickly. The plasma proteins in 1 ml. samples of adrenal venous plasma were precipitated with an equal volume of 3½ % perchloric acid; 0·2 ml. duplicates of the supernatant were used in the assay without further purification and the pH of the final solution for oxidation was maintained above 6·0 by the addition of 2·8 ml. 1 M acetate buffer (pH 6·5). Arterial plasma samples, treated in the same way, were used to determine background fluorescence; known amounts of adrenaline and noradrenaline were routinely added to 1 ml. aliquots of this plasma to provide internal standards for the assay.

The accuracy and reliability of the method was tested as follows: (1) adrenaline or noradrenaline in amounts ranging from 5 to 500 ng were added to 1 ml. aliquots of arterial plasma; recovery was virtually 100 % over the whole range tested (Fig. 1); (2) the recovery of unequal mixtures of adrenaline and noradrenaline was tested by adding different amounts of adrenaline to three concentrations of noradrenaline in plasma (20, 50, and 100 ng). Fig. 2 shows that the recovery of both amines was 90–100 %, irrespective of the adrenaline: noradrenaline ratio except when 10 times the amount of adrenaline was added to the lowest concentration of noradrenaline.

The lower limit of the method was determined by the blank fluorescence due to the reagents and to non-specific fluorescence of the arterial plasma. Catecholamine concentrations of 10 ng/ml. were only 10 % above the blank readings; this precluded the measurement of changes in resting output with any accuracy.

Statistical analyses were made according to the methods of Snedecor & Cochran (1967).

RESULTS

Responses to moderate insulin hypoglycaemia

Endocrine responses to moderate hypoglycaemia were examined in six 2–5 week old calves given 0·1 u. insulin/kg body wt. as a single I.V. injection at time = 0 (Figs. 3, 4). The outputs of both cortisol and corticosterone from the right adrenal gland were found to rise in response to hypoglycaemia during these experiments and peak values coincided with the nadir in plasma glucose concentration. The concentration of cortisol but not corticosterone in the peripheral plasma also rose, reflecting the increase in output from the gland but the change was not statistically significant due to wide individual variation. Maximal steroid outputs were found to be associated with elevated adrenal blood flow which exhibited a transient rise when plasma glucose concentration was lowest (Figs. 3, 4*b*). This response occurred in the absence of any change in either heart rate or mean aortic pressure.

Administration of insulin at this dose usually produced a transient fall in plasma glucagon concentration followed by a rise between 30 and 40 min. In some animals a very small rise in adrenaline output occurred between 30 and 60 min but no change in noradrenaline output was detected during any of these experiments. The maximum mean adrenaline output for the group was found to be $2{\cdot}1 \pm 0{\cdot}8$ ng kg^{-1} min^{-1}, 35 min after insulin.

The wide variation between individuals given the same dose of insulin is in part attributable to different initial plasma glucose concentrations and

consequential differences in the severity of hypoglycaemia. This is illustrated in Fig. 4 in which the individual values from the two extreme examples are shown.

In the first animal (Fig. 4*a*), resting plasma glucose concentration was comparatively high (87 mg/100 ml.) and never fell below 46 mg/100 ml. at any stage. This stimulus failed to produce any detectable change in catecholamine output or adrenal blood flow although a rise in plasma glucagon concentration was observed. Cortisol and corticosterone output were

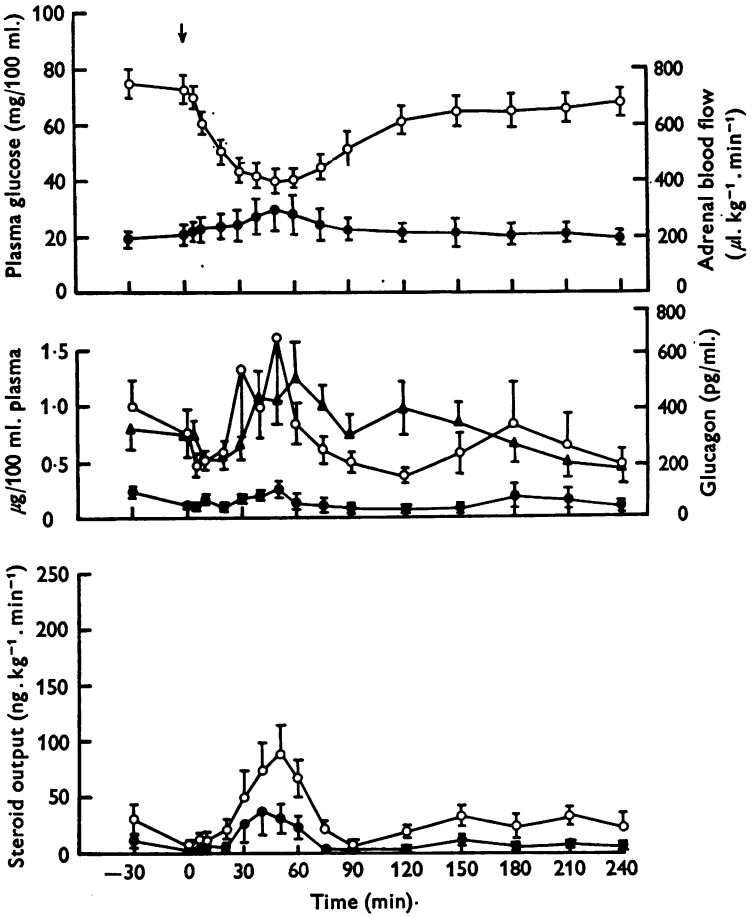

Fig. 3. Mean responses to I.V. insulin (0·1 u./kg) in six calves 2–5 weeks after birth. Above: changes in plasma glucose concentration (○) and right adrenal blood flow (●). Centre: changes in the concentration of glucagon (▲), cortisol (○) and corticosterone (●) in arterial plasma. Below: changes in the output of cortisol (○) and corticosterone (●) from the right adrenal gland. Vertical bars: S.E. of each mean value. Insulin was injected at the arrow.

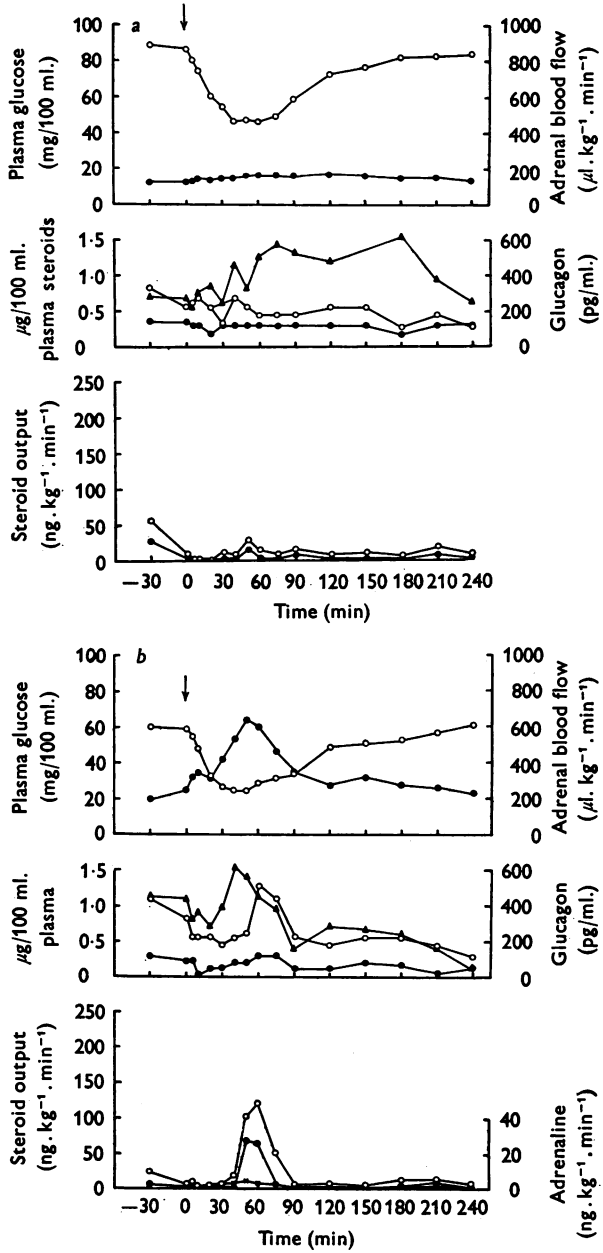

Fig. 4. Responses to I.V. insulin (0·1 u./kg) in two individual calves (*a*, *b*) 2–5 weeks after birth. Above: changes in plasma glucose concentration (○) and right adrenal blood flow (●). Centre: changes in the concentration of glucagon (▲), cortisol (○) and corticosterone (●) in arterial plasma. Below: changes in the output of cortisol (○), corticosterone (●) and adrenaline (×) from the right adrenal gland. Insulin was injected at the arrow.

raised only at 50 min and at no stage increased above levels encountered under control conditions, or by an amount sufficient to raise the concentrations of either steroid in peripheral plasma.

In contrast, plasma glucose concentration fell to much lower levels (25 mg/100 ml.) in the second animal, in which the concentration prior to insulin was also lower (59 mg/100 ml.). Under these conditions, the same dose of insulin was found to produce a substantial increase in the output of both steroids and in adrenal blood flow, together with a detectable rise in adrenaline output (Fig. 4*b*).

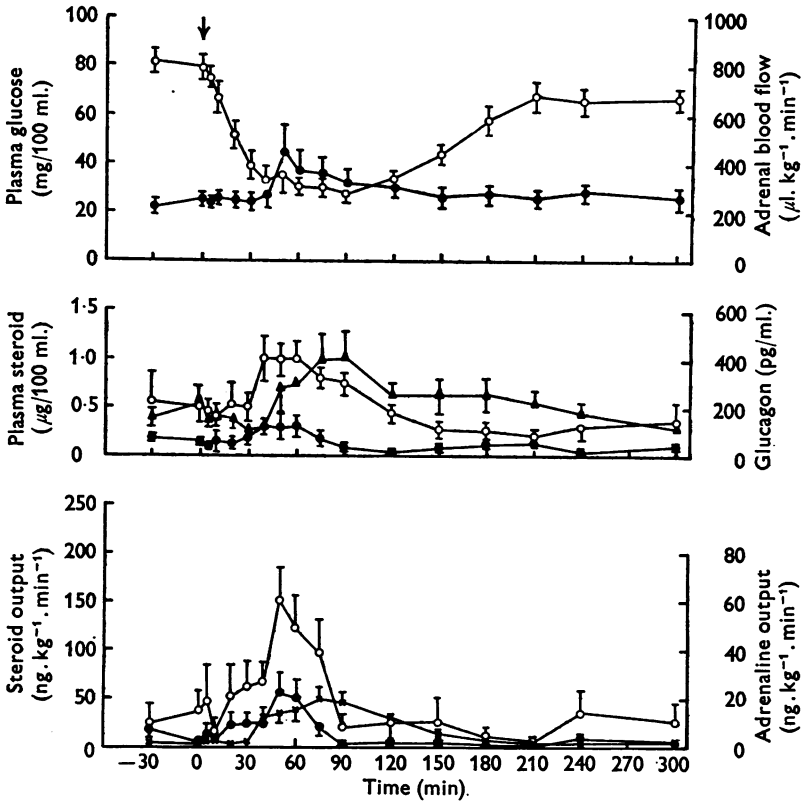

Fig. 5. Mean responses to I.V. insulin (0·5 u./kg) in six calves 2–5 weeks after birth. Above: changes in plasma glucose concentration (○) and right adrenal blood flow (●). Centre: changes in the concentration of glucagon (▲), cortisol (○) and corticosterone (●) in arterial plasma. Below: changes in the output of cortisol (○), corticosterone (●) and adrenaline (×) from the right adrenal gland. Vertical bars: S.E. of each mean value. Insulin was injected at the arrow.

Responses to severe but transient hypoglycaemia

Severe insulin hypoglycaemia was produced in six 2–5-week-old calves by administration of 0·5 u.insulin/kg body wt. (Fig. 5). Mean plasma glucose concentration fell from 79 ± 5 to 33 ± 5 mg/100 ml. within 40 min and then, more slowly, to 28 ± 4 mg/100 ml. at 90 min; thereafter it recovered slowly and was found to be within 14% of the initial value at 210 min. Mean cortisol and corticosterone output rose to higher peak values than previously (153 ± 33 and 56 ± 19 ng.kg^{-1} min^{-1} respectively) but these occurred at the same time (50 min) as in animals given 0·1 u. insulin/kg. Furthermore, the output of both steroids had subsided to within the normal range by 90 min, at which time plasma glucose was lowest. This short-lived increase in cortisol and corticosterone was accompanied by an abrupt rise in mean adrenal blood flow and was associated with elevated concentrations of both steroids in the peripheral plasma (Fig. 5). No significant change in heart rate or in mean aortic blood pressure was observed during the experiments.

In contrast to the adrenal cortical responses, adrenaline output and plasma glucagon concentration rose more slowly to peak values coincident with the occurrence of the lowest plasma glucose concentrations. Both subsided slowly as plasma glucose rose (Fig. 5). Increased adrenaline output was usually accompanied by a small but detectable rise in the output of noradrenaline. No clinical signs which might be attributable to hypoglycaemia were observed in any of these animals or in those given the smaller dose of insulin.

Presentation of mean data from experiments with protocols such as these is misleading if maximum effects occur at different times in different individuals, particularly if the effects are short-lived. Individual responses of two animals from this group serve to illustrate the rapidity with which changes in cortisol and corticosterone output occur during hypoglycaemia under these conditions (Fig. 6). It can also be seen that a maintained hypoglycaemic stimulus produces only a transient increase in steroid output.

Responses to severe and persistent hypoglycaemia

Severe persistent hypoglycaemia was produced in nine 2–5 week old calves by the administration of 4 u.insulin/kg. The subsequent fall in plasma glucose concentration was closely similar to that in calves given 0·5 u./kg except that hypoglycaemia was a little more intense and persisted until the experiment was terminated after 300 min.

The mean output of both cortisol and corticosterone rose in response to hypoglycaemia and elevated values were maintained for a longer period

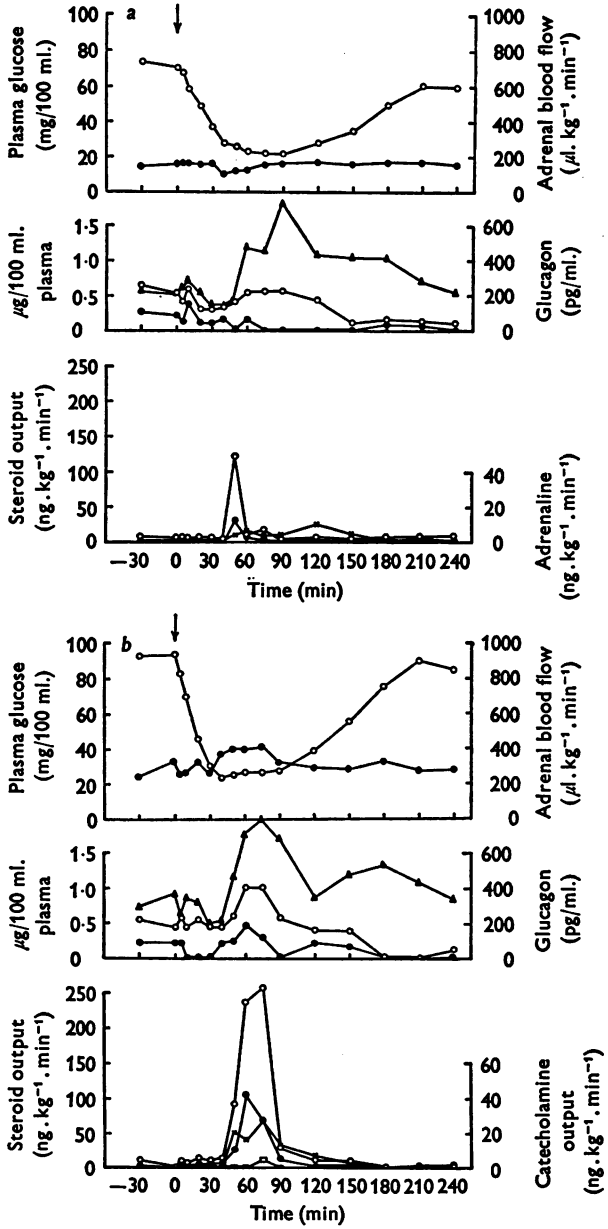

Fig. 6. Responses to I.V. insulin (0·5 u./kg) in two individual calves (*a*, *b*) 2–5 weeks after birth. Above: changes in plasma glucose concentration (○) and right adrenal blood flow (●). Centre: changes in the concentration of glucagon (▲), cortisol (○) and corticosterone (●) in arterial plasma. Below: changes in the output of cortisol (○), corticosterone (●), adrenaline (×) and noradrenaline (□) from the right adrenal gland. Insulin was injected at the arrow.

under these conditions. However, both steroid output and peripheral plasma concentration declined steadily after 120 min in spite of the protracted hypoglycaemia. Mean adrenal blood flow rose steadily, after a delay, from a value of 236 ± 26 μl. kg^{-1} min^{-1} at 20 min to a maximum of 413 ± 51 μl. kg^{-1} min^{-1} at 180 min and was maintained at this comparatively high level thereafter (Fig. 7). In contrast to the changes in

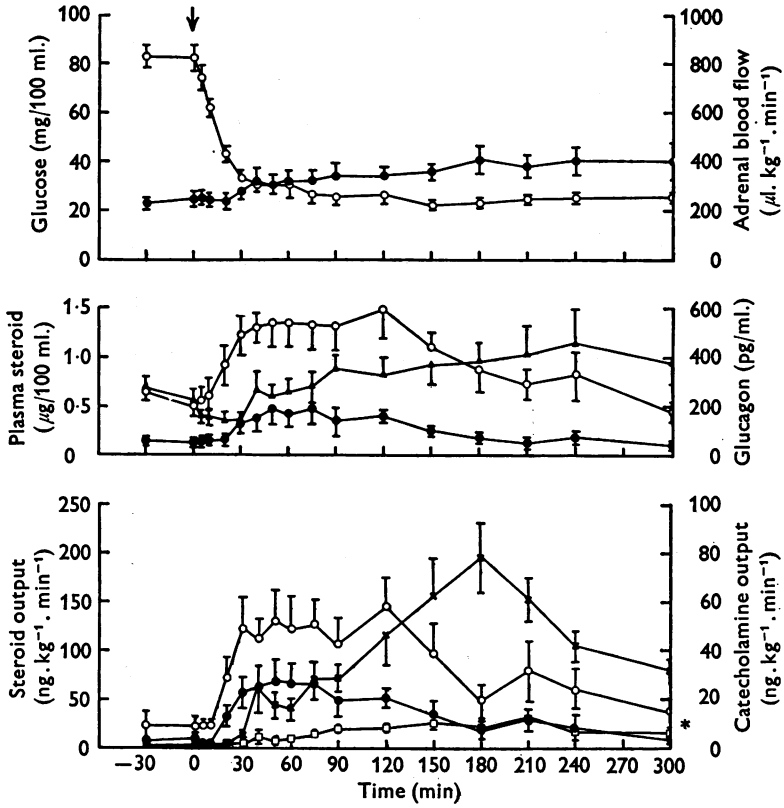

Fig. 7. Mean responses to I.V. insulin (4·0 u./kg) in nine calves 2–5 weeks after birth. Above: changes in plasma glucose concentration (○) and right adrenal blood flow (●). Centre: changes in the concentration of glucagon (▲), cortisol (○) and corticosterone (●) in arterial plasma. Below: changes in the output of cortisol (○), corticosterone (●), adrenaline (×) and noradrenaline (□) from the right adrenal gland. Asterisk represents noradrenaline output above which interference due to adrenaline is negligible. Vertical bars: S.E. of each mean value. Insulin was injected at the arrow.

adrenal blood flow described previously, interpretation of this response is complicated by substantial changes in sympathetic activity and various circulatory parameters (see Fig. 9). This intense hypoglycaemic stimulus

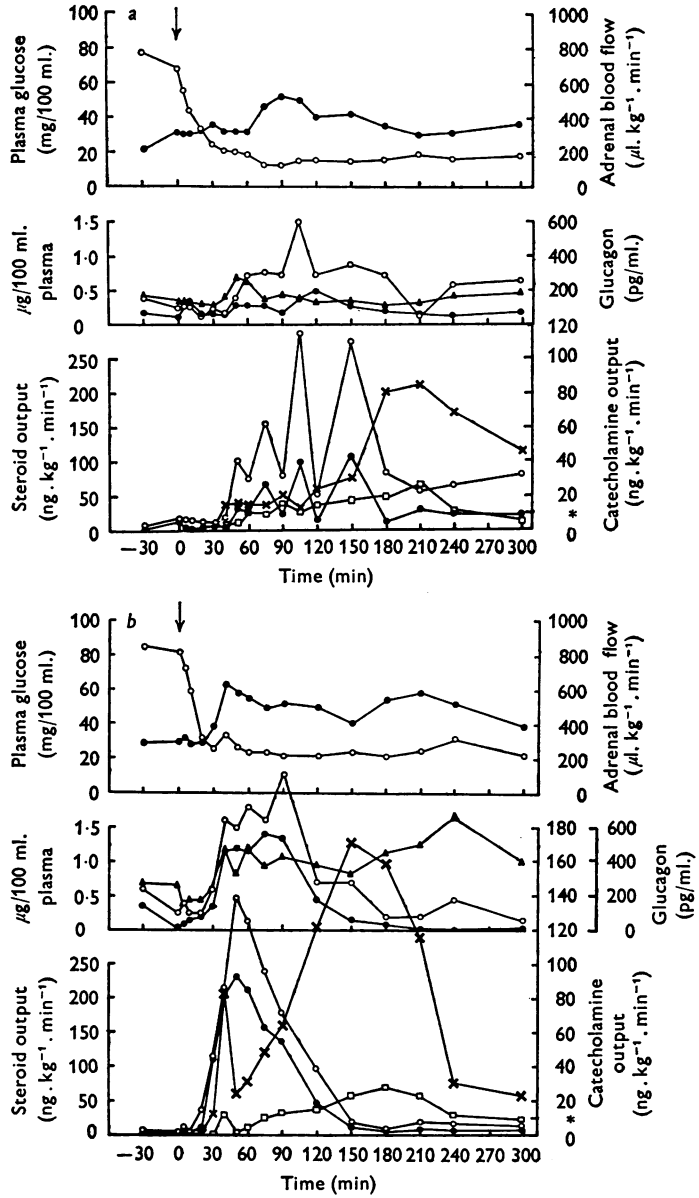

Fig. 8. Responses to I.V. insulin (4·0 u./kg) in two individual calves (*a*, *b*) 2–5 weeks after birth. Above: changes in plasma glucose concentration (○) and right adrenal blood flow (●). Centre: changes in the concentration of glucagon (▲), cortisol (○) and corticosterone (●) in arterial plasma. Below: changes in the output of cortisol (○), corticosterone (●), adrenaline (×) and noradrenaline (□) from the right adrenal gland. Asterisk represents noradrenaline output above which interference due to adrenaline is negligible. Insulin was injected at the arrow.

produced a substantial rise in adrenaline output accompanied by a much smaller release of noradrenaline. The maximum adrenal medullary response occurred later than that of the cortex; adrenaline and noradrenaline outputs were both rising as steroid output subsided and adrenal medullary activity was often maximal when steroid output had fallen to within the normal range (Figs. 7, 8). It is likely that mean noradrenaline outputs observed represent an underestimate, due to interference effects consequent upon the high concentrations of adrenaline in these samples (see Fig. 2); each of the mean values for noradrenaline output lies below the

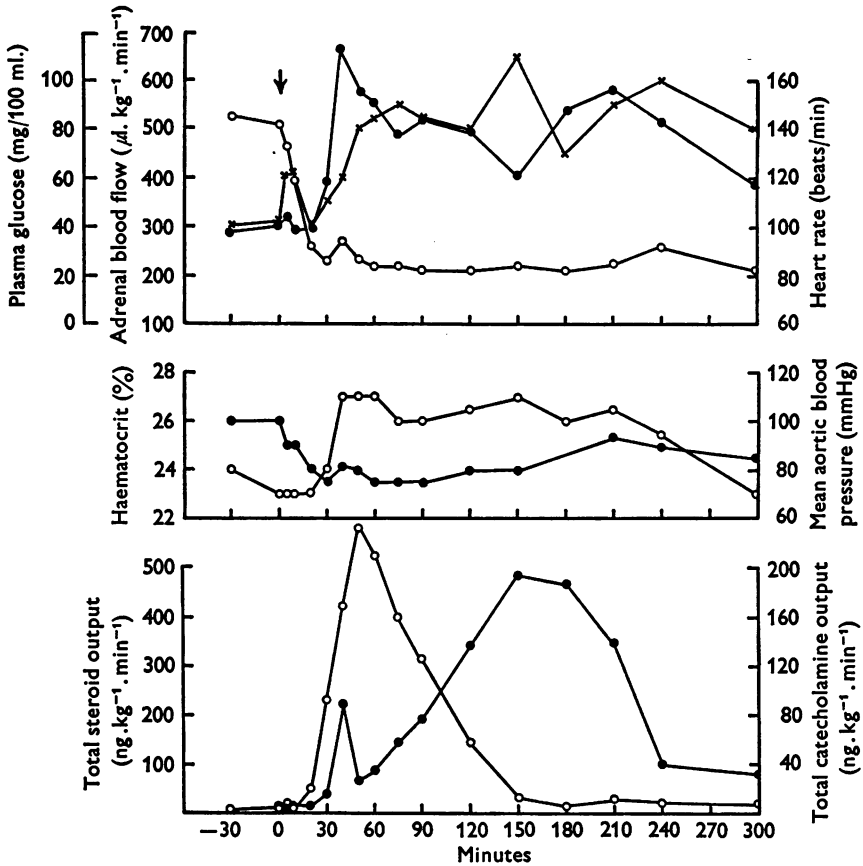

Fig. 9. Responses during hypoglycaemia in a 19-day old calf given 4 u. insulin/kg (identical experiment to that shown in Fig. 8*b*). Above: changes in arterial plasma glucose concentration (○), right adrenal blood flow (●) and heart rate (×). Centre: changes in haematocrit (○) and mean aortic blood pressure (●). Below: changes in total glucocorticoid (○) and catecholamine (●) output from the right adrenal gland. Insulin was injected at the arrow.

level at which such interference must be presumed (Fig. 7). Nevertheless, results from individual animals, in which the concentration of noradrenaline in the adrenal effluent plasma was sufficiently high to ensure accurate estimates, show that large amounts of adrenaline are released from the adrenal medulla under these conditions, but comparatively small amounts of noradrenaline (Fig. 8).

Whereas wide individual variation was encountered in this group of animals, as in the others, each individual exhibited the same pattern of response described above. Thus, in each case there was evidence of a rapid adrenal cortical response, which was not sustained, followed by increased adrenal medullary activity.

TABLE 1. Changes in mean blood haematocrit during insulin hypoglycaemia

	Dose of insulin (u./kg)		
Time (min)	0·1 (n = 6)	0·5 (n = 6)	4·0 (n = 9)
−30	0·3 ± 0·4	0·5 ± 0·4	0·1 ± 0·3
0	0 —	0 —	0 —
5	0·2 ± 0·2	0·2 ± 0·3	−0·3 ± 0·3
10	0·2 ± 0·5	0·4 ± 0·2	−0·2 ± 0·1
20	0·1 ± 0·4	0·1 ± 0·4	−0·1 ± 0·2
30	0·0 ± 0·6	0·0 ± 0·2	−0·4 ± 0·3
40	0·2 ± 0·5	0·0 ± 0·3	0·3 ± 0·6
50	0·9 ± 1·2	0·3 ± 0·7	1·0 ± 0·5
60	1·4 ± 0·9	0·8 ± 0·3	1·9 ± 0·6
75	0·1 ± 0·6	1·3 ± 0·5	1·4 ± 0·6
90	−0·3 ± 0·4	1·5 ± 0·5	1·3 ± 0·6
120	−0·4 ± 0·6	0·3 ± 0·3	2·4 ± 1·0
150	−0·3 ± 0·5	0·0 ± 0·4	1·6 ± 0·8
180	−0·3 ± 0·6	−1·1 ± 0·4	1·4 ± 0·7
210	−0·8 ± 0·5	−0·8 ± 0·3	1·3 ± 0·7
240	−0·8 ± 0·7	−1·4 ± 0·4	0·6 ± 0·7
300	— —	−0·4 ± 0·9	−0·3 ± 0·6

All values are expressed as a change from the value at time = 0 for ease of comparison.

Mean plasma glucagon concentration was found to fall initially and then to rise steadily after 30 min to approximately 400 pg/ml. at 180 min at which level it was maintained (Fig. 7). It would therefore appear under these conditions of severe hypoglycaemia that the response of the pancreatic α cell more closely resembles the response of the adrenal medulla than that of the adrenal cortex.

The majority of these animals collapsed and convulsed 90–180 min after insulin. Convulsions were extremely violent but could be abolished by the administration of a small amount of sodium pentobarbitone (5–6 mg/kg).

This dose of pentobarbitone is equivalent to one fifth the full anaesthetic dose and had no discernible anaesthetic effect when given to normal calves of the same age. Furthermore, each of the experimental animals recovered almost immediately when given intravenous glucose (0·3 g/kg) and consumed 3–4 pints of milk from a bucket after the experiment had been concluded. Neither the occurrence of convulsions nor the administration of barbiturate could be related to any consistent change in endocrine response.

In contrast to the other groups, hypoglycaemia in calves given 4 u. of insulin usually produced a slight rise in haematocrit (Table 1). This coincided with the increasing output of catecholamines (Fig. 7 and Table 1) and in some animals was accompanied by substantial increase in heart rate (Fig. 9). No consistent change in blood pressure was observed, so that this would not explain the persistence of increased adrenal blood flow after steroid output had subsided to low values.

DISCUSSION

The results of these experiments provide direct confirmatory evidence that the adrenal cortical response to hypoglycaemia is more sensitive than that of the adrenal medulla. A significant increase in the output of both cortisol and corticosterone was found to occur in response to a moderate fall in plasma glucose concentration, which produced barely detectable changes in catecholamine output. Furthermore, secretion of glucocorticoids was initiated earlier, during severe hypoglycaemia, than secretion of catecholamines. Measurements of the changes in concentration of glucocorticoids in peripheral plasma in the human suggest that differences in the sensitivity of the adrenal cortical response to this stimulus between species are comparatively small. Thus, Bertrand, Loras & Frederich (1962) demonstrated a significant increase in plasma 17 OH corticosteroid concentration during hypoglycaemia following the administration of 0·1 u. insulin/kg in the human infant and no such response is observed in the adult until the blood glucose concentration falls below 40 mg/100 ml. (Landon, Wynn & James, 1963). More recent studies using radioimmunoassay techniques to measure plasma adrenocorticotrophin have provided ample confirmation of this earlier work (Landon & Greenwood, 1968; Donald, 1971; Ichikawa, Nishikai, Kawagoe, Yoshida & Homma, 1972; Staub, Jenkins, Ratcliffe & Landon, 1973).

Secretion of glucocorticoids during hypoglycaemia depends upon the release of adrenocorticotrophin from the anterior pituitary (Gershberg & Long, 1948; Steeples & Jensen, 1949). In the present series of experiments, increased steroid output was associated with a rise in adrenal blood flow,

together with a fall in the ratio of cortisol: corticosterone released from the gland in each group of animals. This is in precise accordance with the known effects of the trophin in this species (Table 2; Edwards *et al.* 1974, 1975), for, as the dose of exogenous adrenocorticotrophin is increased within the physiological range, the proportion of corticosterone secreted progressively rises to a maximum. In the present experiments reduction of the cortisol: corticosterone ratio was most pronounced in those animals in which hypoglycaemia was most intense and glucocorticoid output was highest (4 u. insulin/kg; Table 2).

TABLE 2. Changes in mean cortisol: corticosterone ratio (adrenal output) during insulin hypoglycaemia

	Dose of insulin (u./kg)		
Time (min)	0·1 (n = 6)	0·5 (n = 6)	4·0 (n = 9)
Prior to infusion	3·2 ± 0·6	3·0 ± 0·7	3·1 ± 0·7
20–40	4·0 ± 0·5	2·4 ± 0·4	2·1 ± 0·2
60	4·5 ± 1·1	3·7 ± 1·6	2·4 ± 0·5
120	5·1 ± 0·9	2·8 ± 0·9	2·9 ± 0·4
180	3·8 ± 0·8	4·2 ± 1·7	3·8 ± 0·9
240	3·9 ± 0·5	4·3 ± 1·2	3·1 ± 0·5
300	—	3·5 ± 1·1	2·6 ± 0·8

The adrenal cortical response to hypoglycaemia was of limited duration, even when the stimulus was prolonged (4 u. insulin/kg; Fig. 7), in agreement with Zukoski's findings in the anaesthetized dog (Zukoski, 1966). The observation that the proportion of the two glucocorticoids which was released from the gland reverted to normal as the absolute output declined, therefore provides a clue that the short-lived adrenal cortical response merely reflects the transient nature of the pituitary reaction to this stimulus. These glucocorticoids have little or no direct hyperglycaemic effect in normal calves, either when administered intravenously (A. V. Edwards, unpublished observations), or when released in maximal amounts in response to exogenous adrenocorticotrophin (Edwards *et al.* 1975). However, the finding that the pituitary-adrenal cortical axis responds so rapidly to a fall in plasma glucose concentration is in complete accord with the observation that bilateral adrenalectomy rapidly leads to spontaneous hypoglycaemia in the calf at this age (Comline & Edwards, 1965; Edwards, 1970). The evidence presented here further indicates that those who consider that the adrenal cortical response to hypoglycaemia is non-specific (see for instance Marks & Samols, 1968) should reconsider this question. These steroids have long been known to exert a potent gluconeogenic effect (Long & Lukens, 1936; Ashmore & Weber, 1968) and also play a

'permissive' role in the glycogenolytic response to glucagon and the catecholamines (Ingle, 1954).

Comparison of the present results with previous studies in which the adrenal cortical response to different doses of synthetic adrenocorticotrophin was examined in calves of the same age (Edwards *et al.* 1974, 1975) allows an approximate estimate of the amount of endogenous trophin released in response to this stimulus to be made. Moderate hypoglycaemia (0·1 u. insulin/kg) produced changes in adrenal steroid output and blood flow equivalent to infusion of Synacthen at a dose of 0·5 ng.kg^{-1} min^{-1} for a limited time. Severe hypoglycaemia produced adrenal responses which corresponded to a dose of the trophin between 0·5 and 5·0 ng.kg^{-1} min^{-1} for a short period. Even the most intense and prolonged hypoglycaemic stimulus, sufficient to cause collapse and convulsions, produced a relatively small increase in steroid output by comparison with that which occurs in response to administration of very large amounts of exogenous adrenocorticotrophin (600–800 ng cortisol.kg^{-1} min^{-1} during infusion of Synacthen at 50 ng.kg^{-1} min^{-1}; Edwards *et al.* 1974*b*). It may therefore be concluded that the limiting factor during hypoglycaemia is the amount of pituitary trophin released, rather than the capacity of the adrenal cortex to respond.

The classic study by Cannon, McIver & Bliss (1924) originally demonstrated release of sympathin from the adrenal medulla in response to severe hypoglycaemia. Subsequent studies have shown that this stimulus only becomes effective when the blood glucose concentration falls below a certain critical level (Armin & Grant, 1959), or the supposed central nervous gluco-receptors are effectively blocked by other means (Hökfelt & Bydgeman, 1961; Himsworth, 1968*a*, *b*), and strongly suggests that there is a selective release of adrenaline under these conditions (Hökfelt, 1951; Euler & Luft, 1952; Duner, 1954; Crone, 1965). The young calf would appear to be an ideal species in which to examine the proposition that release of noradrenaline and adrenaline from the adrenal medullae may be selective, since non-specific chemical or electrical stimulation under anaesthesia produces a secretion in which noradrenaline is the predominant amine (Silver, 1960; Comline & Silver, 1966).

The fact that the proportionate release of the two amines differs so markedly when the response to hypoglycaemia is compared with that to maximal stimulation of the adrenal innervation, substantiates the view that results of acute experiments cannot be extrapolated to predict responses in conscious animals. In the present experiments, both blood haematocrit and heart rate were elevated when catecholamine output was evident, suggesting that increased adrenal medullary output reflected generalized increase in sympathetic efferent activity. This contention is

supported by the observation that increase in catecholamine output was accompanied by a rise in plasma glucagon concentration, since the pancreatic alpha cell is extremely sensitive to stimulation via the sympathetic innervation (Bloom *et al.* 1973).

Whereas the disparity between the sensitivities of the two adrenal responses to hypoglycaemia is obvious, it is more difficult to assess the sensitivity of the pancreatic response. Thus, peak plasma glucagon concentrations occurred at 50–60 min during moderate hypoglycaemia (0·1 u.insulin/kg) as found previously (Bloom *et al.* 1974); this was approximately coincident with the peak steroid output. This initial response was absent in calves given larger amounts of insulin, although the plasma glucagon concentration rose later, at the time that adrenal medullary activity was apparent. In view of the fact that both divisions of the autonomic nervous system influence the release of this hormone (Bloom *et al.* 1973, 1974) it seems likely that initial release of glucagon during moderate hypoglycaemia mediated by the parasympathetic innervation is relatively sensitive to insulin inhibition (Marks & Samols, 1968) and thus becomes completely suppressed when larger doses of insulin are employed. The delayed release of glucagon during severe hypoglycaemia probably represents part of the generalized sympathetic response to severe stress and is not inhibited, even after administration of these highly unphysiological amounts of insulin (4 u./kg).

One of the most striking features of the adrenal response to hypoglycaemia is the apparent reciprocity of glucocorticoid and catecholamine output. The fact that maximal activity of the adrenal medulla occurs later than that of the cortex can be explained on the basis of differences in the sensitivity of the two systems to this stimulus. However, the transient nature of the adrenal cortical response to persistent hypoglycaemia raises the question as to whether or not activity of the pituitary–adrenal cortical axis is susceptible to some form of adrenergic inhibition. Intravenous infusions of adrenaline in man, in similar amounts to those released endogenously in the calf during severe hypoglycaemia, have been found to suppress the rise in plasma cortisol concentration after insulin (Müller-Hess, Geser, Jéquier, Felber & Vannotti, 1974). However, these results could as well be explained by the rise in blood glucose concentration produced by adrenaline under these conditions. Other workers have found that the increase in plasma glucocorticoid concentration in response to adrenocorticotrophin is reduced by simultaneous administration of catecholamines (Sandberg, Nelson, Palmer, Samuels & Tyler, 1953; Moor Hinnekens, Steeno, Deckx, Delaere & Meulepas, 1962), suggesting direct adrenergic inhibition of the cortical response to the trophin. It is intended to provide a more precise evaluation of such interactions in the future.

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