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ADRENAL AND PANCREATIC ENDOCRINE RESPONSES TO HYPOXIA IN THE CONSCIOUS CALF

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SUMMARY

1. Pancreatic and adrenal responses to intense hypoxia have been examined in conscious unrestrained calves 3–5 weeks after birth.

2. The outputs of both cortisol and corticosterone from the right adrenal gland rose steadily in response to hypoxia and this cortical secretory response was accompanied by a pronounced increase in blood flow through the gland. The changes in both steroid output and adrenal blood flow corresponded with those which occur in response to supramaximal doses of corticotrophin in calves of the same age.

3. Neither adrenaline nor noradrenaline were released in significant amounts from the adrenal medulla until the arterial P_{O_2} had fallen below 15 mmHg. Such severe hypoxia caused secretion of catecholamines at rates comparable with those recorded during maximal stimulation of the sympathetic innervation to the gland in anaesthetized calves. The response to intense hypoxia in these conscious calves differed from that which occurs under anaesthesia in that the amount of adrenaline released was invariably greater than that of noradrenaline.

4. Severe hypoxia produced a rapid but transient increase in plasma glucagon concentration, followed by a pronounced rise in plasma glucose concentration in animals with abundant liver glycogen. No change in plasma insulin concentration was observed during hypoxia although it rose subsequently in response to hyperglycaemia.

5. Bilateral section of the splanchnic nerves virtually abolished the release of catecholamines in response to hypoxia but the adrenal cortical and pancreatic responses did not appear to be affected.

INTRODUCTION

Studies of adrenal medullary function in anaesthetized calves during the first few weeks after birth have shown that noradrenaline is the predominant amine to be released in response to such diverse stimuli as hypoxia, acetyl choline or maximal electrical stimulation of the splanchnic nerves (Silver, 1960; Comline & Silver, 1966). In contrast, severe hypoglycaemia elicits the secretion of proportionately greater amounts of adrenaline in conscious calves of the same age (Bloom, Edwards, Hardy, Malinowska & Silver, 1975). Preferential release of adrenaline in response to hypoglycaemia would appear to be biologically advantageous as the remarkable resistance of the new-born calf to severe hypoglycaemia has been shown to depend upon the release of adrenaline, but not noradrenaline, from the adrenal medulla (Edwards, 1964; Comline & Edwards, 1968). However, the apparent discrepancy between results obtained in conscious and in anaesthetized animals might well be due to anaesthetic interference with catecholamine release. In the present experiments this question has been examined by assessing the adrenal medullary response to severe hypoxia in conscious calves of the same age as those previously investigated under anaesthesia. In addition the changes in glucocorticoid output from the adrenal cortex and in the release of pancreatic hormones have been measured in order to provide a more comprehensive analysis of endocrine changes during severe hypoxia in the conscious animal.

Some of the observations have been published previously in a preliminary form (Bloom, Edwards, Hardy, Malinowska & Silver, 1976).

METHODS

Animals. Pedigree Jersey calves were obtained from local farms shortly after birth and used at ages ranging from 21 to 33 days (24·1–35·4 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 hypoxia was induced. 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 hypoxia was initiated 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. When required both splanchnic nerves were cut immediately below the diaphragm. Procaine penicillin (600,000 i.u.) and dihydrostreptomycin (0·5 g) (May and Baker Ltd, Dagenham) were administered routinely before 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 nitrogen was administered, in order to accustom the animals to the sampling procedure. During this period the animals were habituated to wear a light loosely-fitting 'helmet' which was constructed of aluminium and transparent polyethylene. This fabrication measured 29 × 29 × 54 cm and was perfused with air at a rate of 15 l./min. 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.

At time = 0, nitrogen was substituted for air and perfused through the 'helmet' at the same rate. Samples of arterial and right adrenal effluent blood were collected at intervals, during and after hypoxia. Adrenal blood flow was estimated gravimetrically and corrected for haematocrit % before the output of steroids and catecholamines from the gland was computed. The period for which the animals would tolerate hypoxia varied between 8 and 10 min and the final mean values during hypoxia have therefore been 'normalized' to a notional 9 min value. Statistical analyses were made according to the methods of Snedecor & Cochran (1967).

Analytical procedures. Arterial blood samples were collected anaerobically for blood gas and pH estimations and into heparinized tubes containing aprotonin (Trasylol: Bayer; 1000 k i.u./ml. blood) for other analyses. These tubes were centrifuged at +4° C immediately and the plasma then stored at −20° C.

Glucagon was measured by a radio-immunoassay using an antiserum relatively specific for pancreatic glucagon which was C terminal reacting (Assan & Slusher, 1972); this assay reacted less than 5% with glucagon like immunoreactivity of ileal origin (enteroglucagon). Insulin was also measured by radio-immunoassay (Albano, Ekins & Turner, 1972) and glucose was estimated using a Beckman Glucose Analyser. Cortisol and corticosterone were measured by competitive protein binding (Malinowska, Hardy & Nathanielsz, 1972).

Blood P_{O_2}, P_{CO_2} and pH were measured, immediately after samples had been collected, using standard Radiometer equipment equilibrated at 38·5° C.

Adrenal venous blood samples were analysed for cortisol and corticosterone, as above, and also for adrenaline and noradrenaline by a modification of Euler & Floding's trihydroxyindole method (Euler & Floding, 1955) as previously described (Bloom *et al.* 1975).

At the conclusion of each experiment small pieces of liver were removed for glycogen analysis (Edwards, 1971).

RESULTS

Mean responses to intense hypoxia

Intense hypoxia was induced in six conscious, unrestrained calves, 3–5 weeks after birth, by perfusing nitrogen at a rate of 15 l./min through a light, transparent 'helmet' placed over the heads of the animals. Fig. 1*A* shows the changes in mean pH, P_{O_2} and P_{CO_2} of the arterial blood which occurred in response to this procedure. Arterial P_{O_2} fell abruptly during the first 2 min and both the rate and depth of respiration rose rapidly.

These changes were accompanied by a fall in arterial P_{CO_2} and the expected rise in blood pH (Fig. 1*A*). Mean arterial P_{O_2} continued to fall during the next 2 min and very low levels persisted thereafter until air was substituted for nitrogen at between 8 and 10 min. By this time the animals had lost consciousness and spontaneous respiration had ceased. Each animal

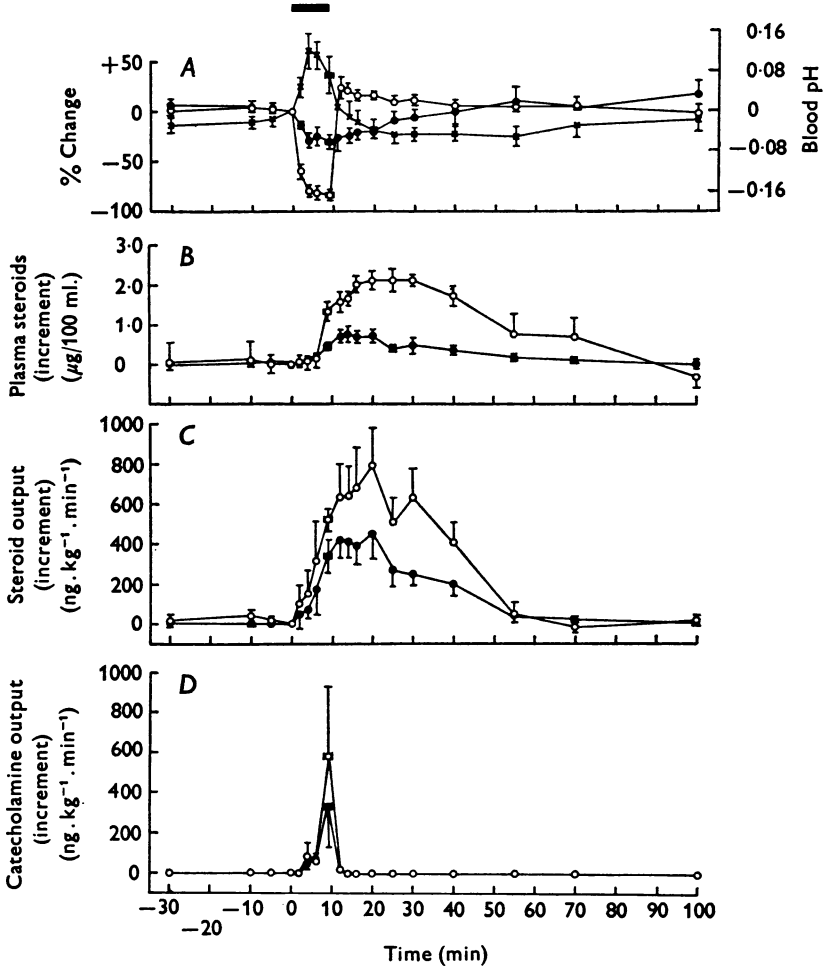

Fig. 1. Responses to intense hypoxia in six 3–5-week-old calves. *A*, changes in mean arterial P_{O_2} (○), P_{CO_2} (●) and blood pH (×); *B*, mean arterial plasma cortisol (○) and corticosterone (●) concentrations; *C*, mean cortisol (○) and corticosterone (●) outputs from the right adrenal gland; *D*, mean adrenaline (○) and noradrenaline (●) outputs from the right adrenal gland. Horizontal bar: duration of hypoxia (8–10 min). Vertical bars: S.E. of each mean value where this exceeds the size of the symbol.

recovered rapidly, however, in response to vigorous artificial ventilation for a few seconds, while air was once more perfused through the 'helmet' at the same rate (15 l./min).

The outputs of both cortisol and corticosterone increased steadily during hypoxia and had risen by 522 ± 64 ng.kg^{-1}.min^{-1} and 339 ± 80 ng.kg^{-1}.min^{-1} ($n = 6$) respectively when the stimulus was withdrawn at between 8 and 10 min. Maximal outputs (cortisol: 741 ± 174 ng.kg^{-1}.min^{-1}; corticosterone: 450 ± 121 ng.kg^{-1}.min^{-1}) were not achieved, however, until 10 min after the hypoxic stimulus had been discontinued. Thereafter, the outputs of both steroids subsided steadily and had fallen to within the resting range by 70 min (Fig. 1*C*). The time course of the changes in the concentrations of cortisol and corticosterone in the arterial plasma (Fig. 1*B*) support the presumption that these were consequential upon the changes in the rate of secretion of the two steroids.

In contrast to the adrenal cortical secretory response, substantial release of catecholamines from the adrenal medulla did not occur until the very end of hypoxia (Fig. 1*D*). The comparatively large standard error at the time of peak secretion (adrenaline: 581 ± 349 ng.kg^{-1}.min^{-1}; noradrenaline: 332 ± 200 ng.kg^{-1}.min^{-1}; $n = 6$) was due to large differences in the absolute amounts of the two amines secreted by different individuals. In other respects the responses of these animals were closely similar. Thus, neither catecholamine was secreted in substantial amounts until the P_{O_2} of the arterial blood had fallen below 15 mmHg, by which time the animals were *in extremis*. The output of adrenaline at this time invariably exceeded that of noradrenaline and release of both catecholamines ceased abruptly as arterial P_{O_2} rose during recovery.

Mean right adrenal blood flow rose from 215 ± 23 μl.kg^{-1}.min^{-1} at time = 0 to a mean maximum value of 562 ± 67 μl.kg^{-1}.min^{-1} 2 min after hypoxia was discontinued and had subsided to within the resting range by 60 min. Individual animals exhibited wide but transient fluctuations in aortic blood pressure, pulse pressure and heart rate which were particularly pronounced just before and after the stimulus was discontinued. However, the differences in mean aortic blood pressure at the precise times at which samples were collected were comparatively small, indicating that the rise in adrenal blood flow was due to vasodilation of adrenal blood vessels. There was also a pronounced increase in mean haematocrit during hypoxia: both the latency and duration of this response were closely similar to those which characterized the rise in adrenal blood flow.

The pancreatic islets were also found to be extremely sensitive to severe hypoxia and a rapid but transient increase in plasma glucagon concentration was invariably observed (Fig. 2). This was associated with

a pronounced rise in plasma glucose concentration in animals with abundant liver glycogen. In contrast to plasma glucagon, no significant change in plasma insulin concentration occurred during hypoxia, although mean plasma insulin concentration rose subsequently. This later effect was absent in calves with depleted reserves of liver glycogen and in which there was no rise in plasma glucose concentration. It is therefore concluded that the rise in mean plasma insulin concentration which occurred after hypoxia had been discontinued (Fig. 2) represented a secondary response due to hyperglycaemia.

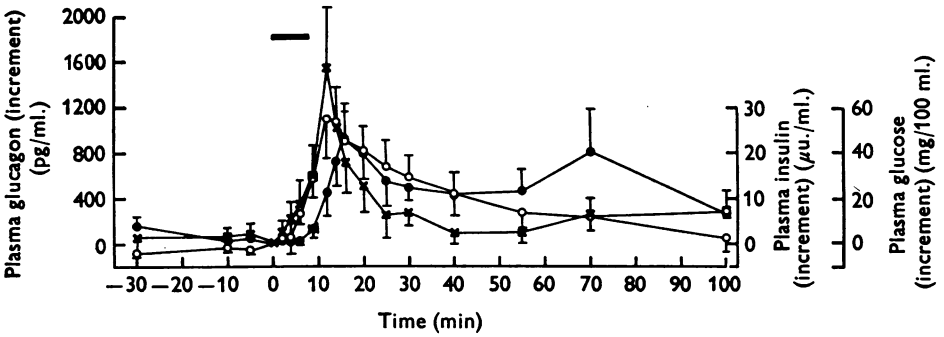

Fig. 2. Comparison of the changes in mean arterial plasma glucagon (×), glucose (○) and insulin (●) concentrations in response to hypoxia in six 3–5-week-old calves. Horizontal bar: duration of hypoxia. Vertical bars: S.E. of each mean value.

Intense hypoxia also provoked a marked increase in plasma lactate concentration (Table 1). No significant change occurred during the first 6 min but a rapid increase was observed between 8 and 14 min and elevated values persisted thereafter during each experiment.

Individual responses to intense hypoxia

The comparatively wide individual variations which were encountered were at least partly due to the fact that the administration of nitrogen through the 'helmet' did not necessarily result in the same degree of hypoxia in each calf. This is illustrated in Fig. 3 in which individual values from the two extreme examples are shown.

In the first animal (Fig. 3*A*) arterial P_{O_2} fell comparatively slowly and was still above 20 mmHg at 10 min when hypoxia was terminated (Fig. 3*A a*). This stimulus failed to produce any detectable change in catecholamine output although there was a pronounced rise in glucocorticoid secretion (Fig. 3*A b*) together with increased blood flow through the right

adrenal gland (Fig. 3*Ac*). The changes in the concentrations of glucagon, insulin and glucose in the plasma were also muted in this experiment.

In contrast, the arterial P_{O_2} fell much more rapidly in the second animal and a value of 8 mmHg was recorded at 10 min, immediately before the stimulus was withdrawn (Fig. 3*Ba*). Hypoxia of this severity elicited a sudden transient discharge of adrenaline (1828 $ng.kg^{-1}.min^{-1}$) together with rather less noradrenaline (724 $ng.kg^{-1}.min^{-1}$) from the adrenal medulla. This response was followed by the release of maximal amounts

TABLE 1. Changes in mean plasma lactate concentration (mg/100 ml.) in response to intense hypoxia (0–10 min) in six calves 3–5 weeks after birth

Time	Mean value	*D*
−30	12·3 ± 2·5	+ 2·1
0	10·2 ± 3·1	0
2	10·3 ± 2·8	+ 0·1
4	15·5 ± 4·4	+ 5·3
6	13·4 ± 3·5	+ 3·2
8	26·2 ± 8·2	+16·0
10	66·7 ± 11·6	+56·4
12	102·1 ± 6·1	+91·9
14	104·3 ± 11·1	+94·1
20	98·6 ± 12·3	+88·4
30	88·3 ± 14·4	+78·1
100	43·1 ± 18·2	+32·9

D, difference from value at time = 0.

of cortisol and corticosterone from the adrenal cortex (Fig. 3*Bb*) and a maximal increase in right adrenal blood flow (Fig. 3*Bc*). It is noteworthy that the heart rate had fallen sharply when hypoxia was terminated (Fig. 3*Bc*; 10 min) in this experiment. Such severe hypoxia also produced a massive increase in plasma glucagon concentration during recovery when it rose from 365 pg/ml. at 10 min to a peak value of 1950 pg/ml. at 12 min. This was just preceded by a rise in plasma glucose concentration and followed by a much slower increase in plasma insulin concentration (Fig. 3*Bd*).

Responses to intense hypoxia after section of both splanchnic nerves

The effects of intense hypoxia were also examined under the same conditions in calves in which both splanchnic nerves had been cut 24 hr previously and the results of two such experiments are illustrated in Fig. 4. These animals were found to be more susceptible to hypoxia as judged by the cardiovascular changes and it was therefore necessary to terminate the stimulus a little earlier in this group (5–8 min). Release of both adrenaline

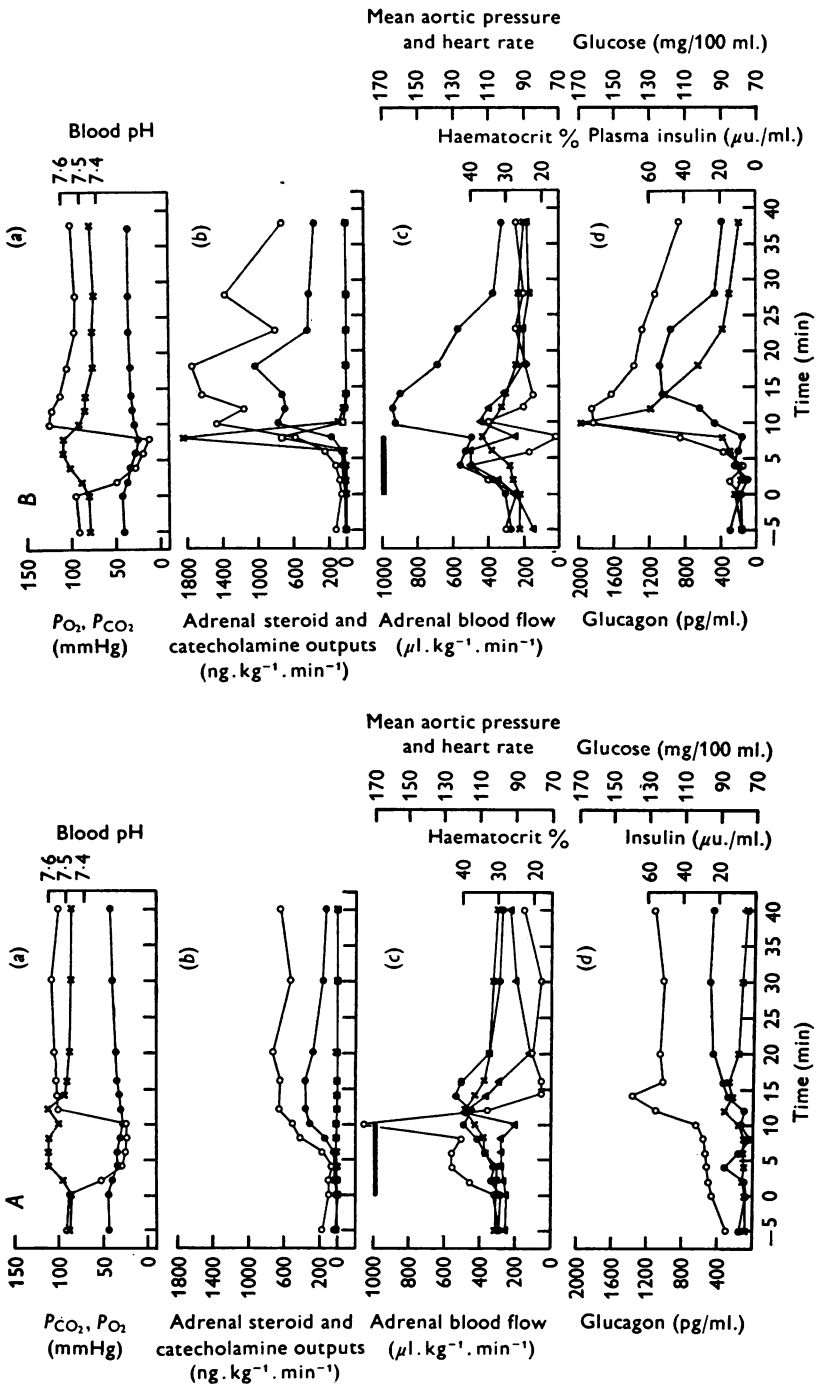

Fig. 3. Responses to hypoxia in two individual calves (*A*, *B*) 3–5 weeks after birth: (*a*) arterial P_{O_2} (○), P_{CO_2} (●) and blood pH (×); (*b*) outputs of cortisol (○), corticosterone (●), adrenaline (×) and noradrenaline (□) from the right adrenal gland; (*c*) right adrenal blood flow (●), haematocrit (×), mean aortic blood pressure (▲) and heart rate (○); (*d*) plasma glucagon (×), insulin (●) and glucose (○) concentrations. Horizontal bar: duration of hypoxia (10 min).

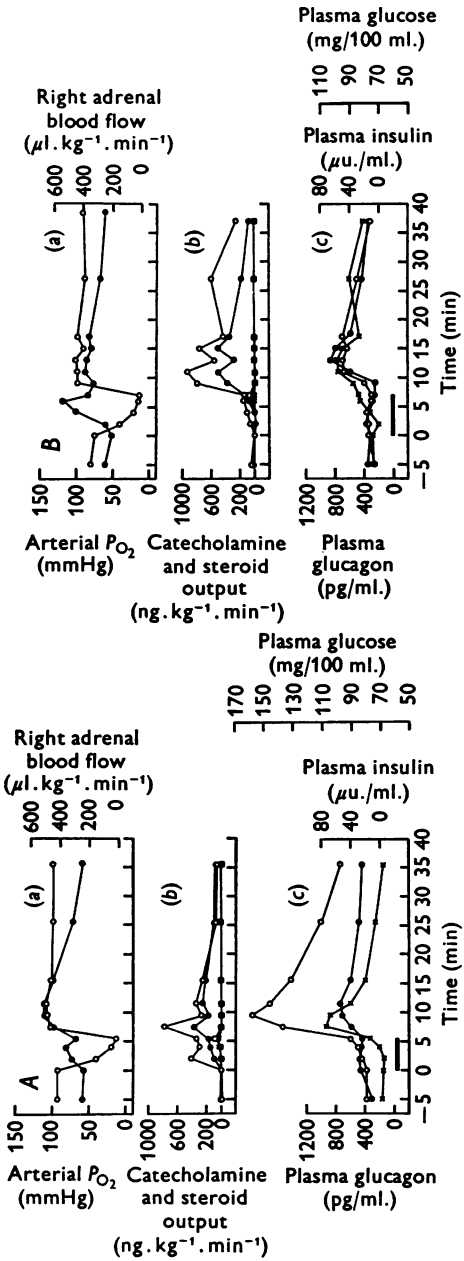

Fig. 4. Responses to hypoxia in two individual calves (*A*, *B*) 3–5 weeks after birth after section of both splanchnic nerves: (*a*) arterial P_{O_2} (○) and right adrenal blood flow (●); (*b*) outputs of cortisol (○), corticosterone (●), adrenaline (□) and noradrenaline (×) from the right adrenal gland; (*c*) plasma glucagon (×), insulin (●) and glucose (○) concentrations. Horizontal bars: duration of hypoxia.

and noradrenaline from the adrenal medulla was virtually abolished in all these experiments (Fig. 4*A*, *B*). However, neither the adrenal cortical secretory response nor the rise in adrenal blood flow were affected by section of the splanchnic nerves. Hypoxia was also associated with a rise in plasma glucagon concentration and this was accompanied by pronounced hyperglycaemia in animals with abundant liver glycogen (Fig. 4*B*).

DISCUSSION

The rapidity with which the outputs of cortisol and corticosterone from the right adrenal gland rose during hypoxia shows that the pituitary–adrenal axis is extremely sensitive to a reduction of arterial P_{O_2}. This observation is in general accord with finding of other workers who have demonstrated a rise in the concentrations of corticotrophin or glucocorticoids in response to hypoxia in a variety of species (Hirai, Atkins & Marotta, 1963; Marks, Battacharya & Vernikos-Danellis, 1965; Moncloa, Donayre, Sobrevilla & Guerra-Garcia, 1965; Lau, 1971; Boddy, Jones, Mantell, Ratcliffe & Robinson, 1974). Comparison of the peak rates of glucocorticoid release (cortisol: 741 ± 174 ng.kg^{-1}.min^{-1}; corticosterone: 450 ± 121 ng.kg^{-1}.min^{-1}) and right adrenal blood flow (562 ± 67 μl.kg^{-1}.min^{-1}) in the present experiments, with those observed in response to infusions of exogenous corticotrophin, indicates that intense hypoxia elicits a maximal secretory response from the adrenal cortex, accompanied by maximal vasodilation within the gland. Maximal cortisol and corticosterone outputs in response to exogenous corticotrophin fell within the range 600–800 ng.kg^{-1}.min^{-1} and 350–500 ng.kg^{-1}.min^{-1} respectively and right adrenal blood flow increases by between 200 and 500 μl.kg^{-1}.min^{-1} under the same conditions (Edwards *et al.* 1975). The adrenal responses to intense hypoxia in the present experiments were consistent with the changes which would occur in response to a secretion of corticotrophin at a rate of 50 ng.kg^{-1}.min^{-1} for between 10 and 20 min and were unaffected by adrenal denervation.

The adrenal medulla was found to be much more resistant to hypoxia. Significant release of catecholamines was never observed until the arterial P_{O_2} had fallen below 15 mmHg and invariably ceased abruptly as the P_{O_2} rose during recovery. Thus, the adrenal medullary response to hypoxia in the conscious calf resembles that to hypoglycaemia (Bloom *et al.* 1975) in that it can only be elicited by prolonged and intense stimulation. A further similarity between these responses in the conscious animal is that greater amounts of adrenaline than noradrenaline are released from the gland. In this respect the response of the conscious calf differs from that of anaesthetized animals of the same age, in which noradrenaline is the

predominant amine released in response to various stimuli including hypoxia (Silver, 1960; Comline & Silver, 1966). This discrepancy can probably be ascribed to the fact that release of catecholamines from the adrenal medulla is modified by anaesthesia. The type of anaesthetic has been shown to have a pronounced effect on the absolute amounts of adrenaline and noradrenaline released in response to asphyxia in calves of this age; much higher outputs of both noradrenaline and adrenaline are achieved in calves anaesthetized with chloralose than in animals under pentobarbitone anaesthesia (Comline & Silver, 1966). Total catecholamine output at the end of hypoxia in the present experiments (913 $ng.kg^{-1}.min^{-1}$) was similar to that released in response to maximal stimulation of the splanchnic innervation under chloralose anaesthesia (1060 $ng.kg^{-1}.min^{-1}$ at 2–3 weeks, $n = 8$; 1030 $ng.kg^{-1}.min^{-1}$ at 4–5 weeks, $n = 7$) (Silver, 1960) even though the proportions of adrenaline and noradrenaline were different. It is therefore concluded that although the adrenal medulla is comparatively resistant to hypoxia, a sufficiently intense stimulus is capable of eliciting a maximal response from both the adrenal cortex and medulla.

The changes in the concentrations of pancreatic glucagon and insulin in the arterial plasma in response to hypoxia indicate that there is a rapid release of glucagon from the pancreas followed, after a delay, by release of insulin. Both release of glucagon and inhibition of insulin release are known to occur in response to stimulation of the sympathetic innervation in the calf at this age (Bloom, Edwards & Vaughan, 1973) and adrenergic stimulation may have contributed to the changes found in the present experiments. However, very similar pancreatic responses occurred in animals with cut splanchnic nerves, showing that these changes cannot be attributed solely to such adrenergic effects. Release of pancreatic glucagon also occurs in response to cholinergic stimulation both in calves and dogs (Bloom, Edwards & Vaughan, 1974; Kaneto & Kosaka, 1974; Kaneto, Miki & Kosaka, 1974) but the results of preliminary experiments have shown that a substantial rise in plasma glucagon and insulin concentration occur in response to hypoxia in atropinized calves with cut splanchnic nerves (S. R. Bloom, A. V. Edwards and R. N. Hardy, unpublished). It is therefore possible that the pancreatic α and β cells are directly sensitive to low arterial P_{O_2} in these animals.

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