J. Physiol. (1977), **269**, *pp.* 131–154
With 12 *text-figures*
Printed in Great Britain

ADRENAL AND PANCREATIC ENDOCRINE RESPONSES TO HYPOXIA AND HYPERCAPNIA IN THE CALF

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(*Received 2 December 1976*)

SUMMARY

1. Adrenal and pancreatic endocrine responses to hypoxia and hypercapnia, of differing degrees of intensity, have been examined in conscious, unrestrained calves 3–5 weeks after birth.

2. The outputs of cortisol and corticosterone from the right adrenal gland were found to vary inversely with arterial P_{O_2} between 17 and 55 mmHg. Significant increase in mean adrenal blood flow was not observed at arterial oxygen tensions above about 30 mmHg.

3. Release of physiologically effective amounts of catecholamines from the adrenal medulla occurred only in response to intense hypoxia (arterial P_{O_2} $17{\cdot}1 \pm 2{\cdot}8$ mmHg) and was effectively abolished by section of both splanchnic nerves. Release of pancreatic glucagon in response to such intense hypoxia was unaffected by section of both splanchnic nerves and administration of atropine. In contrast, the rise in plasma pancreatic glucagon concentration during less intense hypoxia was abolished by autonomic blockade.

4. Hypercapnia produced by inhalation of either 5% or 10% CO_2 for 30 min stimulated maximal release of adrenal glucocorticoids and caused a substantial rise in plasma glucagon concentration. In contrast, the adrenal medulla was found to be extremely resistant to hypercapnia. Significant release of catecholamines was only observed during intense hypercapnia (inhalation of 10% CO_2) and noradrenaline was invariably found to be the predominant amine.

5. The results of these experiments show how endocrine responses to hypoxia and hypercapnia are graded in the conscious calf. Of the mechanisms we have examined the pituitary–adrenal cortical axis is the most sensitive and the adrenal medulla the most resistant, while the pancreatic α cell occupies an intermediate position.

INTRODUCTION

It is generally accepted that the pituitary–adrenal cortical axis is extremely sensitive to a wide variety of 'stresses' and both hypoxia and hypercapnia are known to cause a rapid increase in the concentrations of corticotrophin and of adrenal glucocorticoids in the circulating plasma (Hirai, Atkins & Marotta, 1963; Marks, Battacharya & Vernikos-Danellis, 1965; Moncloa, Donayre, Sobrevilla & Guerra-Garcia, 1965; Lau, 1971*a*; Boddy, Jones, Mantell, Ratcliffe & Robinson, 1974). It is also widely supposed that the adrenal medulla is similarly responsive to these stimuli. Such a belief appears eminently reasonable in view of the known sensitivity of the sympathetic system to variations in arterial P_{O_2} and P_{CO_2} (see for instance, Folkow & Neil, 1971), given that the gland forms an integral part of that system. However, results of experiments in anaesthetized animals suggest that the adrenal medulla is comparatively resistant to asphyxia (Von Euler & Folkow, 1953; Celander, 1954).

The extent to which the adrenal medulla may participate in responses to 'stresses' that fall within the physiological range can only be assessed by the use of conscious animals. Previous studies in the conscious calf have shown that the adrenal medulla fails to respond to such diverse stimuli as moderate hypoglycaemia (Bloom, Edwards, Hardy, Malinowska & Silver, 1975*a*) or moderate hypoxia (Bloom, Edwards, Hardy & Silver, 1976) and is not implicated in the generalized sympathetic discharge which invariably occurs during feeding (Bloom *et al.* 1975*b*).

In the present experiments this question has been examined in conscious calves, exposed to different intensities of either hypoxia or hypercapnia. In addition, changes in glucocorticoid output and release of pancreatic hormones have been monitored in an attempt to evaluate the relative sensitivities of these endocrine responses to respiratory stresses.

Certain of these results have been published previously in a preliminary form (Bloom, Edwards, Hardy & Malinowska, 1977).

METHODS

Animals

Pedigree Jersey calves were obtained from local farms shortly after birth and used at ages ranging from 21 to 34 days (23·8–42·5 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 experiments. 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 or the concentration of

pancreatic glucagon in the arterial plasma was found to be elevated above the normal resting range at the start of an experiment 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 rhythm. In each case, samples of adrenal effluent and/or arterial blood were collected at 30 min intervals for 2–3 hr before the experiment, 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 the composition of gas perfused through the 'helmet' was altered as shown below

Hypoxia grade 1	7·5 l. N_2/min	7·5 l. compressed air/min
Hypoxia grade 2	3·5 l. N_2/min	11·5 l. compressed air/min
Hypoxia grade 3	1·75 l. N_2/min	13·25 l. compressed air/min

In all the above experiments a small muslin bag filled with soda lime was placed in the helmet to absorb CO_2.

Hypercapnia grade 1	1·5 l. CO_2/min	13·5 l. compressed air/min
Hypercapnia grade 2	0·75 l. CO_2/min	14·0 l. compressed air/min

The experimental gas mixture was perfused through the helmet from 0 to 30 min, after which time the compressed air flow was restored. Samples of arterial and right adrenal blood were taken before, during and after the experimental period as shown below.

−30, −10, −5, 0, 5, 10, 15, 20, 30, 35, 40, 45, 50, 60, 75, 90, 120, 150 min.

When required, intense hypoxia was induced by substituting N_2 for air completely and perfusing it through the 'helmet' at the same rate (15 l./min). The period for which the animals tolerated this stimulus varied between 8 and 10 min and the final mean values have therefore been adjusted to a notional 9 min value (Figs. 11 and 12).

In some experiments atropine (atropine sulphate, B.D.H.) was given by I.V. injection (0·9 % (w/v) NaCl, 0·1 g/100 ml.) at a dose of 0·2 mg/kg 10 min before hypoxia was initiated.

Adrenal blood flow was estimated gravimetrically and corrected for haematocrit % before the output of steroids and catecholamines from the gland was computed. 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 glucagon and insulin analyses. Blood samples were centrifuged at +4° C immediately and the plasma then stored at −20° C.

Glucagon was measured by a radioimmunoassay 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 radioimmunoassay (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) and the estimated values include correction for column recovery.

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*a*). Allowance has been made for arterial plasma steroid concentration in computing adrenal steroid output.

At the conclusion of each experiment small pieces of liver were removed for glycogen analysis (Edwards, 1971). These estimations established that the concentration of glycogen in the liver exceeded 10 mg/g in each animal in this series.

RESULTS

Cardiovascular and respiratory responses to hypoxia

A total of thirteen calves were exposed to hypoxia for a period of 30 min. Of these, seven were tested with a gas mixture comprising 7·5 l. N_2/min and 7·5 l. air/min (designated grade 1 hypoxia), four were given 3·5 l. N_2/min and 11·5 l. air/min (designated grade 2 hypoxia) and two were given 1·75 l. N_2/min and 13·25 l. air/min (designated grade 3 hypoxia).

The consequential changes in arterial P_{O_2} (Fig. 1*A*) provide an index of the intensity of the hypoxic stimulus. During grade 1 hypoxia arterial P_{O_2} fell abruptly to less than half the initial value within 5 min and more gradually thereafter to reach a steady level (20·3 ± 1·8 mmHg; *ca.* 25% of the initial value) between 20 and 30 min. During grade 2 hypoxia arterial P_{O_2} fell along a similar, but less steeply inclined curve to a final value of 29·8 ± 1·5 mmHg (*ca.* 40% of the initial value). In the two individuals exposed to grade 3 hypoxia arterial P_{O_2} fell to 59·8 and 42·5 mmHg respectively.

The changes in arterial P_{CO_2} and pH which occurred during grade 1 and 2 hypoxia (Fig. 1*B*, *C*) presumably reflect increased pulmonary ventilation. Recovery from hypoxia was invariably rapid and all three

parameters had returned to normal 15 min after the stimulus was withdrawn. Grade 3 hypoxia produced little or no significant change in either blood pH or P_{CO_2}.

Apart from a small, statistically insignificant, rise in mean aortic blood pressure initially, during grade 1 hypoxia, mean blood pressure remained comparatively stable during all these experiments (Table 1); transient variations in individual animals could not be related to the hypoxic

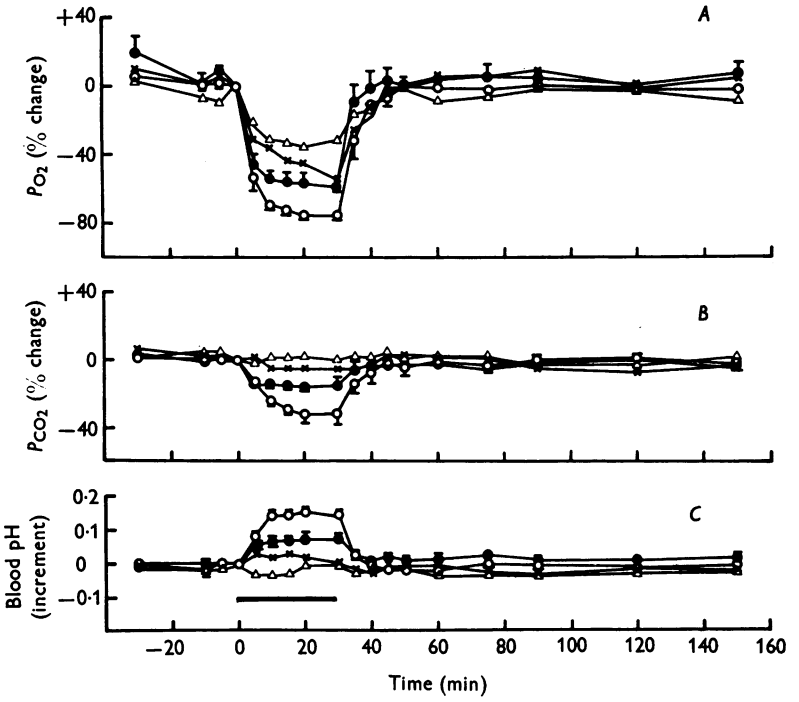

Fig. 1. Comparison of the changes in arterial P_{O_2}, P_{CO_2} and blood pH in groups of 3–5 week old calves in response to grade 1 (○; n = 7) and grade 2 (●; n = 4) hypoxia. Also in two individual animals of the same age in response to grade 3 hypoxia (△, ×). Vertical bars: S.E. of each mean value where visible. Horizontal bar: duration of stimulus.

stimulus with any consistency. In contrast, grade 1 hypoxia caused pronounced tachycardia within 5 min and an elevated heart rate persisted until the stimulus was withdrawn. A rapid increase in respiratory rate, to values approximately double those at rest, was also observed in these animals and there was a slight but persistent rise in haematocrit. Grade 2 hypoxia caused a slight rise in mean respiratory rate, together with mild tachycardia, but no change in mean haematocrit (Table 1). Grade 3 hypoxia was without apparent effect on any of these parameters.

TABLE 1. Comparison of the changes in mean aortic blood pressure, heart rate, haematocrit and respiratory rate in 3–5 week old calves in response to grade 1 ($n = 7$) or grade 2 ($n = 4$) hypoxia for 30 min

Time (min)	Aortic blood pressure (mmHg)				Heart rate (beats/min)				Haematocrit (%)				Respiratory rate (breaths/min)			
	Grade 1		Grade 2		Grade 1		Grade 2		Grade 1		Grade 2		Grade 1		Grade 2	
		D		*D*		*D*		*D*		*D*		*D*		*D*		*D*
−10	92·2±6·5	−7·5	96·6±7·6	−3·4	113·0±10·3	−4·0	121·3±4·7	−1·2	25·5±2·3	−0·6	29·9±2·7	−0·1	35 ±8·7	−2·4	36 ±3·5	−0·3
−5	97·3±8·4	−2·4	100·3±6·5	0·3	112·5±6·5	−4·5	114·3±7·5	−8·2	25·8±2·2	−0·3	29·8±2·5	−0·2	36 ±10·3	−1·4	35·3±4·4	−1·0
0	99·7±6·5	0	100·0±7·5	0	117·0±9·8	0	122·5±4·8	0	26·1±2·3	0	30·0±2·7	0	37·4±5·4	0	36·3±4·0	0
5	107·8±7·05	8·1	100·3±6·9	0·3	158·7±8·7	41·7	130·0±12·7	7·5	26·1±2·4	0	30·0±2·6	0	61·2±10·0	23·8	42·5±1·5	6·2
10	95·5±6·78	−4·2	99·8±7·2	−0·2	141·2±17·8	24·2	130·3±6·3	7·8	27·5±2·9	1·4	29·5±2·1	−0·5	77·7±9·4	40·3	43·3±5·8	7·0
15	90·7±5·92	−9·0	97·3±7·4	−2·7	133·3±19·7	16·3	126·3±5·5	3·8	27·7±3·1	1·6	29·8±2·2	−0·2	83 ±14·2	45·6	40·8±5·8	4·5
20	96·8±6·95	−2·9	97·0±8·7	−3·0	127·5±12·2	10·5	123·8±7·5	1·3	27·5±2·9	1·4	29·6±2·4	−0·4	66·5±8·0	29·1	42·5±5·7	6·2
30	102·3±5·3	2·6	94·0±6·3	−6·0	131·7±9·9	14·7	126·3±5·9	3·8	27·9±2·9	1·8	28·9±2·5	−1·1	67·7±5·9	30·3	42·0±5·8	5·7
35	94·0±4·33	−5·7	91·8±5·8	−8·2	112·5±10·2	−4·5	111·3±5·2	−11·2	26·6±2·7	0·5	28·9±2·5	−1·1	38·3±5·0	0·9	31·0±1·3	−5·3
40	98·0±2·0	−1·7	91·0±3·9	−9·0	125·0±15·0	8·0	122·5±5·9	0	26·1±2·4	0	29·0±2·1	−1·0	39·6±1·6	2·2	30·5±1·9	−5·8

D = difference from the value at time 0.

Adrenal responses to hypoxia

Grade 1 hypoxia caused a rapid rise in the output of both cortisol and corticosterone from the right adrenal gland in each animal. The outputs of the two steroids had increased by $498{\cdot}5 \pm 31{\cdot}8$ and $313{\cdot}9 \pm 28{\cdot}3$ ng.kg^{-1} min^{-1} respectively at 20 min and these rates of secretion were maintained until the stimulus was withdrawn. Thereafter they subsided slowly and

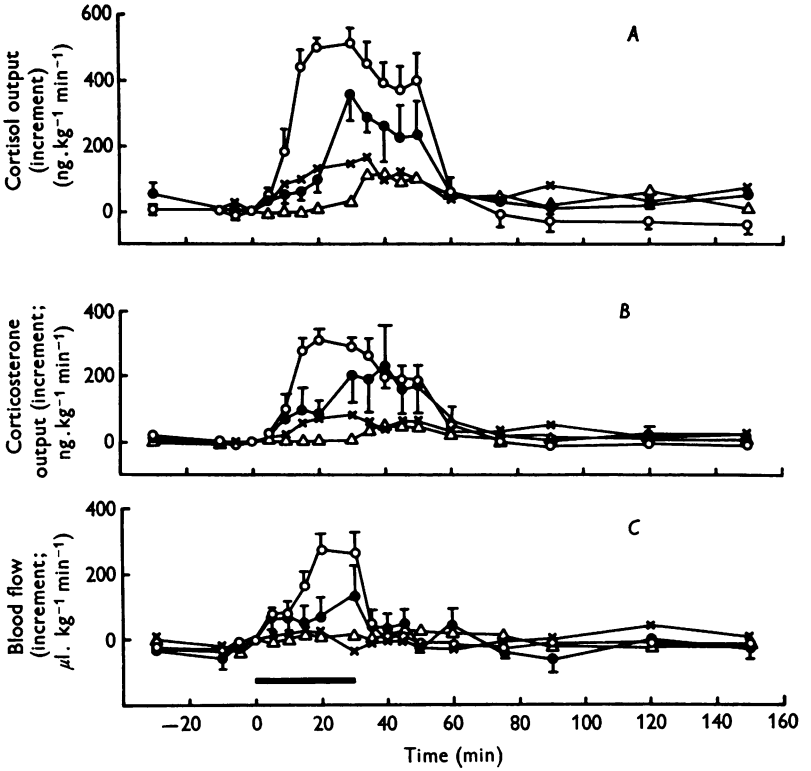

Fig. 2. Comparison of the changes in (*A*) cortisol and (*B*) corticosterone output from the right adrenal gland together with (*C*) right adrenal blood flow in groups of 3–5 week old calves in response to grade 1 (○; $n = 7$) or grade 2 (●; $n = 4$) hypoxia. Also in two individuals of the same age in response to grade 3 hypoxia (△, ×). Vertical bars: S.E. of each mean value. Horizontal bar: duration of stimulus.

had returned to within the resting range 45 min later (Fig. 2*A*, *B*). The cortical secretory response in these animals was accompanied by a substantial rise in adrenal blood flow. In the absence of any significant change in mean aortic blood pressure (Table 1) this can be attributed to the adrenal vasodilator effect of ACTH (Edwards *et al.* 1974, 1975).

Grade 2 hypoxia caused a more gradual rise in the output of both glucocorticoids (Fig. 2*A*, *B*) and peak values (355 ± 80 ng.kg^{-1} min^{-1} cortisol; 203 ± 88 ng.kg^{-1} min^{-1} corticosterone) were not achieved until 30 min, when the stimulus was discontinued (Fig. 2*A*, *B*). In addition, the adrenal blood flow response was correspondingly reduced (Fig. 2*C*). Increased adrenal steroid output was associated with elevated concentrations of cortisol and corticosterone in the peripheral arterial plasma. The changes in mean plasma cortisol and corticosterone concentration were consistent with those expected to result from the changes in output recorded; they were more marked and more persistent in calves exposed to grade 1 hypoxia than to grade 2 or 3.

The maximum mean rates of glucocorticoid release in response to intense hypoxia, induced by infusing the 'helmet' with N_2 alone (Bloom *et al.* 1976) have been compared with those achieved at the end of grade 1, 2 or 3 hypoxia in the present experiments and related to the mean arterial P_{O_2} at the end of the period of hypoxia (Fig. 3). It seems that any arterial P_{O_2} below about 55 mmHg steroid output is inversely related to arterial P_{O_2} and that the relation is roughly linear. Furthermore, grade 1 and grade 2 hypoxia provoked secretion of glucocorticoids at rates approaching the maximal rate at which the adrenal cortex is capable of responding to ACTH (Edwards *et al.* 1974). Mean P_{O_2} values of the gas mixtures within the 'helmet' at the end of hypoxia were $56{\cdot}5 \pm 5{\cdot}4$ (grade 1) and $81{\cdot}9 \pm 6{\cdot}4$ (grade 2), equivalent to the composition of inspired air at *ca.* 8000 and *ca.* 5000 m respectively.

In contrast to the sensitivity of the adrenal cortical secretory response, the adrenal medulla appears to be extremely resistant to this stimulus in the calf at this age. No release of catecholamine was observed during grade 2 or 3 hypoxia and only a trivial response occurred during grade 1 hypoxia (Fig. 4). As noted previously (Bloom *et al.* 1976*a*) significant release of catecholamine from the adrenal medulla is not observed in these animals until the arterial P_{O_2} falls below 15 mmHg (Fig. 3). The mean value shown for catecholamine output during intense hypoxia would be substantially higher with a reduced standard error if animals in which the arterial P_{O_2} remained above 15 mmHg, and in which very little adrenaline or noradrenaline was released, were excluded from the group.

Cardiovascular and respiratory responses to hypercapnia

Responses to hypercapnia were examined in eleven calves exposed to the stimulus for 30 min. Six of these animals were given a gas mixture comprising 1·5 l. CO_2/min and 13·5 l. air/min (grade 1 hypercapnia). The other five were given 0·75 l. CO_2/min and 14·25 l. air/min (grade 2 hypercapnia).

Hypercapnia produced a progressive rise in mean arterial P_{CO_2} which had risen by roughly 300% and 100% respectively at 30 min in response to grade 1 and 2 hypercapnia (Fig. 5*B*). These changes in mean arterial P_{CO_2} were associated with a steady fall in mean blood pH during the same period (Fig. 5*C*). Both parameters had returned to within the resting range 30 min after the stimulus was terminated.

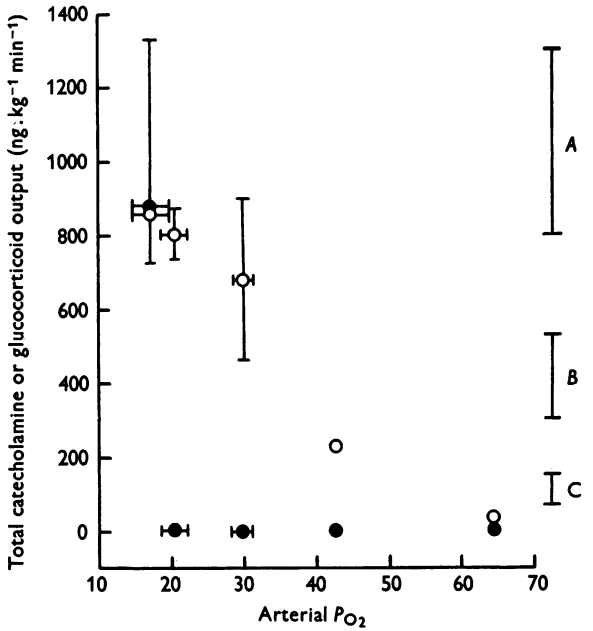

Fig. 3. Relation between total catecholamine (●) and glucocorticoid (○) output from the right adrenal gland in response to hypoxia and mean arterial P_{O_2} at the end of stimulation. Mean maximum outputs obtained in response to intense hypoxia for 10 min (n = 6; Bloom *et al.* 1976) are compared with mean values during grade 1 (n = 7) and grade 2 (n = 4) hypoxia and those from two individuals exposed to grade 3 hypoxia. Horizontal and vertical bars: S.E. of each mean value. *A:* range of glucocorticoid output from the right adrenal gland achieved in response to supramaximal doses of corticotrophin (50 or 500 ng.kg^{-1} min^{-1}) in calves of the same age. *B:* similarly but corresponding to a dose of 5·0 ng.kg^{-1} min^{-1} ACTH. *C:* similarly but corresponding to a dose of 0·5 ng.kg^{-1} min^{-1} ACTH. (Glucocorticoid outputs in response to ACTH computed using data from Edwards *et al.* 1975.)

Hypercapnia caused a substantial increase in the mean respiratory rate in both groups of animals and the effect was most pronounced in response to the less intense stimulus (grade 2, Table 2). In contrast, grade 1 hypercapnia caused a maintained rise in both mean aortic blood pressure and haematocrit, neither of which varied significantly during grade 2

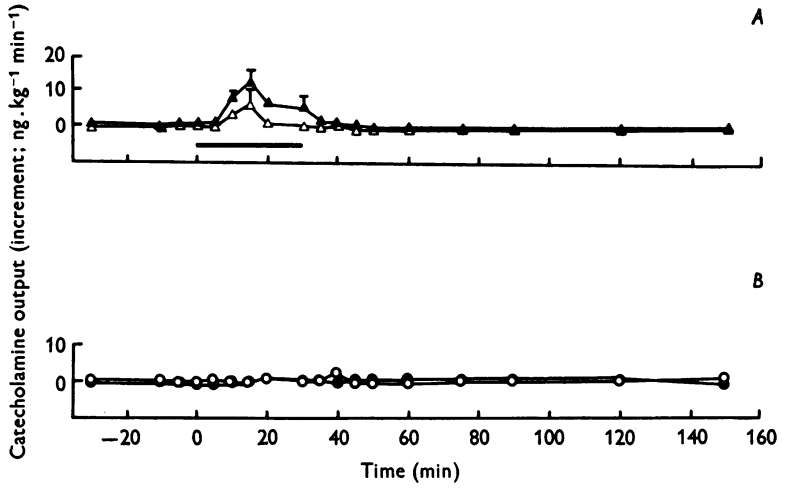

Fig. 4. Comparison of the changes in output of adrenaline (△, ○) and noradrenaline (▲, ●) from the right adrenal gland in groups of 3–5 week old calves in response to grade 1 (△, ▲; $n = 7$) and grade 2 (○, ●; $n = 4$) hypoxia. Vertical bars: S.E. of each mean value where visible. Horizontal bar: duration of stimulus.

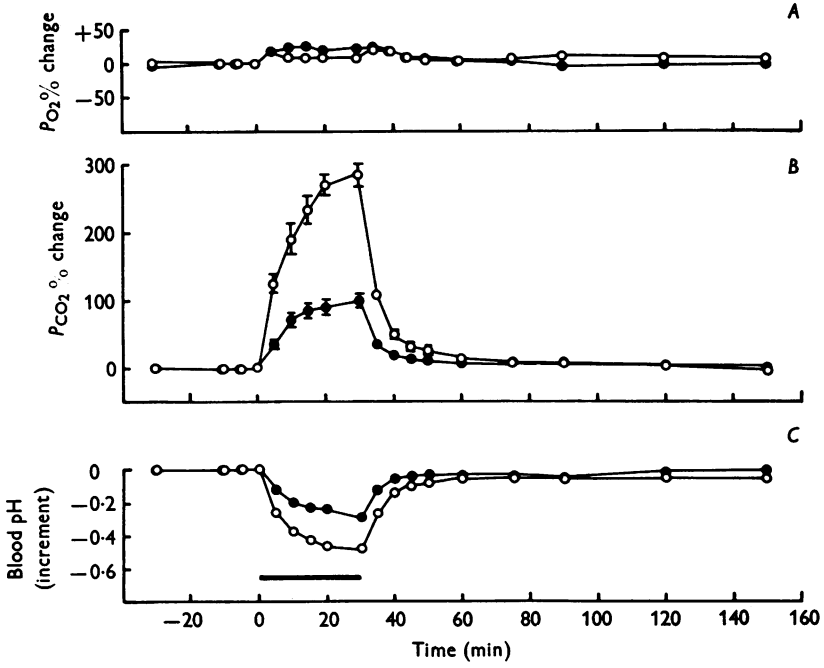

Fig. 5. Comparison of the changes in mean arterial P_{O_2}, P_{CO_2} and blood pH in groups of 3–5 week old calves in response to grade 1 (○, $n = 6$) or grade 2 (●, $n = 5$) hypercapnia. Vertical bars: S.E. of each mean value where visible. Horizontal bar: duration of stimulus.

TABLE 2. Comparison of the changes in mean aortic blood pressure, heart rate, haematocrit and respiratory rate in 3–5 week old calves in response to grade 1 ($n = 6$) or grade 2 ($n = 5$) hypercapnia for 30 min

Time (min)	Aortic blood pressure (mmHg)				Heart rate (beats/min)				Haematocrit (%)				Respiratory rate (breaths/min)			
	Grade 1		Grade 2		Grade 1		Grade 2		Grade 1		Grade 2		Grade 1		Grade 2	
		D		D		D		D		D		D		D		D
−10	102·3±3·7	4·3	95·1±3·2	−2·7	125·5±7·9	2·3	128·6±9·6	−0·8	33·0±4·5	0·6	32·1±2·2	0	29·5±1·9	2·0	29·6±2·0	2·0
−5	97·3±2·0	−0·7	97·6±2·6	−0·2	128·0±5·6	4·8	128·0±9·0	−1·4	33·1±4·9	0·7	32·3±3·2	0·2	28·5±1·7	1·0	30·8±3·4	3·2
0	98·0±2·7	0	98·8±2·7	0	123·2±6·5	0	129·4±7·3	0	32·4±4·4	0	32·1±2·4	0	27·5±2·6	0	27·6±1·8	0
5	102·3±2·5	4·3	95·9±2·2	−1·9	130·0±8·8	6·8	128·1±6·9	−1·3	34·5±4·0	2·1	32·4±2·4	0·3	58·5±2·9	31·0	49·6±4·4	22·0
10	111·5±2·7	13·5	95·9±3·1	−1·9	120·3±16·0	−2·9	131·2±8·3	1·8	35·8±3·9	3·4	32·8±2·2	0·7	59·0±4·1	31·5	73·6±4·8	46·0
15	118·3±6·7	20·3	91·4±4·8	−6·4	123·7±16·7	0·5	126·9±11·3	−2·5	37·0±2·9	4·6	33·1±2·3	1·0	52·5±2·2	25·0	80·0±8·8	52·4
20	116·5±3·0	18·5	90·4±3·8	−7·4	120·0±15·1	−3·2	132·8±8·5	3·4	36·4±3·0	4·0	32·3±2·1	0·2	53·5±3·4	26·0	82·4±11·1	54·8
30	114·7±4·2	16·7	93·3±2·1	−4·5	127·5±17·9	4·3	135·0±9·7	5·6	36·6±2·7	4·2	31·9±2·1	−0·2	49·5±3·6	22·0	73·6±8·6	46·0
35	92·3±3·1	−5·7	87·4±3·0	−10·4	145·0±13·4	21·8	133·8±9·8	4·4	34·5±2·8	2·1	31·1±2·2	−1·0	53·0±3·0	25·5	62·8±5·8	35·2
40	88·8±3·4	−9·2	88·9±1·9	−8·9	135·8±15·8	12·6	135·9±7·7	6·5	32·5±3·2	0·1	30·3±2·0	−1·8	55·3±3·6	27·8	41·2±4·6	13·6

D = difference from the value at time 0.

hypercapnia. No consistent change in heart rate was observed in either group, apart from a transient tachycardia when grade 1 hypercapnia was terminated and when mean blood pressure was falling (Table 2).

Adrenal responses to hypercapnia

The outputs of both cortisol and corticosterone, from the right adrenal gland, rose abruptly in response to hypercapnia in both groups of animals. There was no significant difference between the rates of glucocorticoid secretion achieved which, in both groups, fell within the range previously defined as maximal in response to exogenous corticotrophin (Edwards *et*

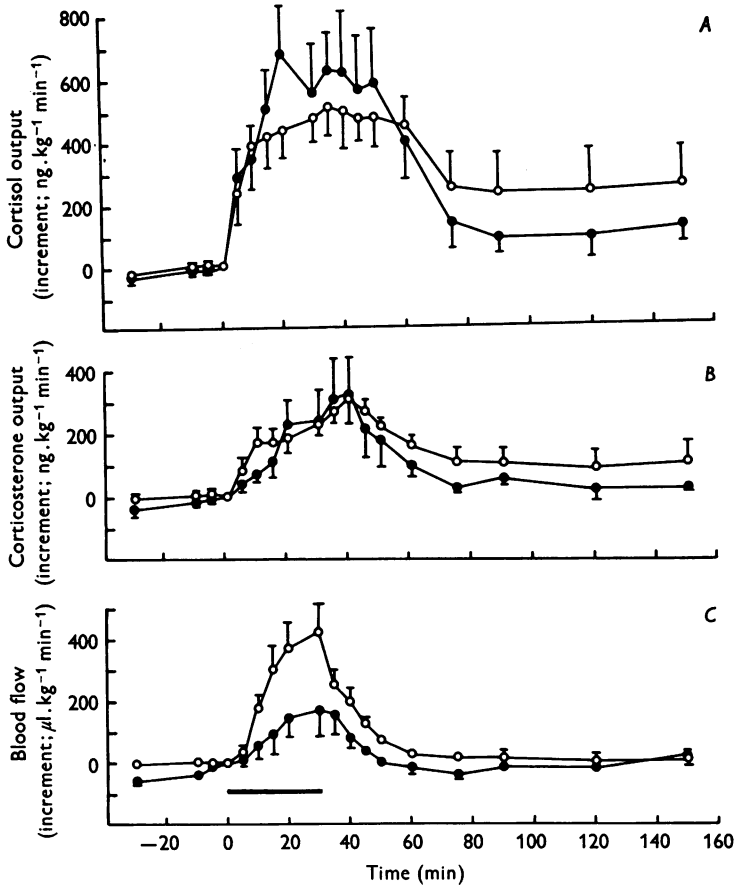

Fig. 6. Comparison of the changes in (*A*) cortisol and (*B*) corticosterone output from the right adrenal gland together with (*C*) right adrenal blood flow in groups of 3–5 week old calves in response to grade 1 (○, $n = 6$) or grade 2 (●, $n = 5$) hypercapnia. Vertical bars: S.E. of each mean value. Horizontal bar: duration of stimulus.

al. 1975; Figs. 3 and 6*A*, *B*). Maximal rates of glucocorticoid secretion were maintained for between 20 and 30 min after withdrawal of the stimulus in both groups, providing further evidence that both grade 1 and 2 hypercapnia probably represent a supramaximal stimulus to the pituitary–adrenal cortical axis. As with hypoxia, the changes in mean arterial plasma cortisol and corticosterone concentrations in all these experiments appeared to reflect the changes in adrenal steroid output. Whereas either grade of hypercapnia produced a similar rate of glucocorticoid secretion, mean adrenal blood flow rose to much higher values during grade 1 than grade 2 (Fig. 6*C*). This difference may be due, at least in part, to the rise in mean aortic blood pressure that occurred during grade 1 hypercapnia (Table 2).

Comparison of the proportion of cortisol:corticosterone secreted by the right adrenal gland (grade 1 hypoxia at time 0: $3{\cdot}58 \pm 0{\cdot}55$; grade 1 hypercapnia at time 0: $2{\cdot}91 \pm 0{\cdot}60$) shows that the ratio falls as the rate of glucocorticoid secretion rises in response to either hypoxia or hypercapnia. Furthermore, closely similar ratios obtained when steroid output was maximal in response to both grade 1 hypoxia and hypercapnia (grade 1 hypoxia at 30 min: $1{\cdot}84 \pm 0{\cdot}11$; grade 1 hypercapnia at 30 min: $1{\cdot}96 \pm 0{\cdot}14$). The proportion of cortisol: corticosterone secreted by the adrenal gland in response to infusions of exogenous ACTH also falls as the dose is raised

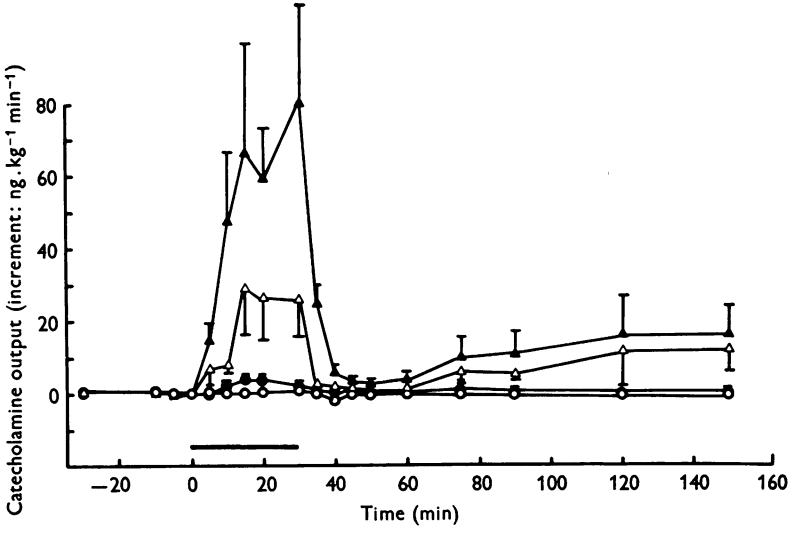

Fig. 7. Comparison of the changes in output of adrenaline (△, ○) and noradrenaline (▲, ●) from the right adrenal gland in groups of 3–5 week old calves in response to grade 1 (△, ▲; $n = 6$) and grade 2 (○, ●; $n = 5$) hypercapnia. Vertical bars: S.E. of each mean value where visible. Horizontal bar: duration of stimulus.

(Edwards *et al.* 1975). The values recorded when the outputs were maximal in the present experiments were closely similar to that found previously in calves of the same age in response to maximal doses of exogenous corticotrophin ($1{\cdot}69 \pm 0{\cdot}15$; ACTH infused at a dose of 50 ng.kg^{-1} min^{-1}).

Hypercapnia was found to be a comparatively ineffective stimulant to catecholamine release from the adrenal medulla. Virtually no medullary response occurred during grade 2 hypercapnia and the peak outputs of catecholamine during grade 1 hypercapnia (noradrenaline: 80 ± 27 ng.kg^{-1} min^{-1}; adrenaline: 26 ± 10 ng.kg^{-1} min^{-1} at 30 min; Fig. 7) are trivial in comparison with those which occur in response to hypoxia if the stimulus is sufficiently intense (Fig. 3). It is noteworthy that the output of noradrenaline invariably exceeded that of adrenaline during hypercapnia whereas adrenaline is the predominant amine released in response to intense hypoxia in the conscious calf (Bloom *et al.* 1976).

Pancreatic responses to hypoxia and hypercapnia

Comparison of the changes in the concentration of pancreatic glucagon in the arterial plasma in response to grade 1 and 2 hypoxia shows that the more intense stimulus provokes release of significant amounts of glucagon from the pancreas whereas the less intense stimulus is ineffective (Fig. 8).

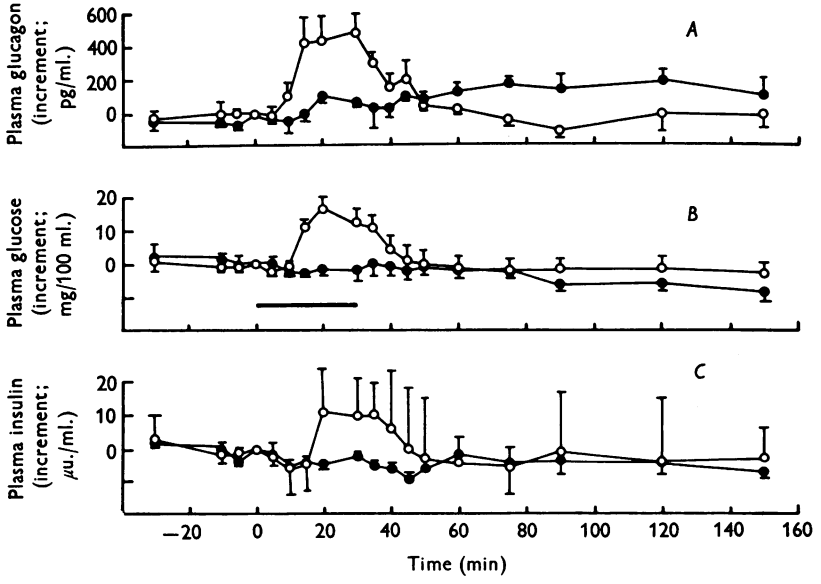

Fig. 8. Comparison of the changes in mean plasma glucagon, glucose and insulin concentration in groups of 3–5 week old calves in response to grade 1 (○, n = 7) or grade 2 (●; n = 4) hypoxia. Vertical bars: S.E. of each mean value. Horizontal bar: duration of stimulus.

The rise in plasma glucagon concentration during grade 1 hypoxia was accompanied by a rise in plasma glucose concentration and followed by release of insulin, both of which responses were absent during grade 2 hypoxia (Fig. 8). It is concluded that the arterial P_{O_2} must fall below about 30 mmHg (30 min value, grade 2 hypoxia) to provide an effective hypoxic stimulus to the release of pancreatic glucagon in the conscious calf at this age.

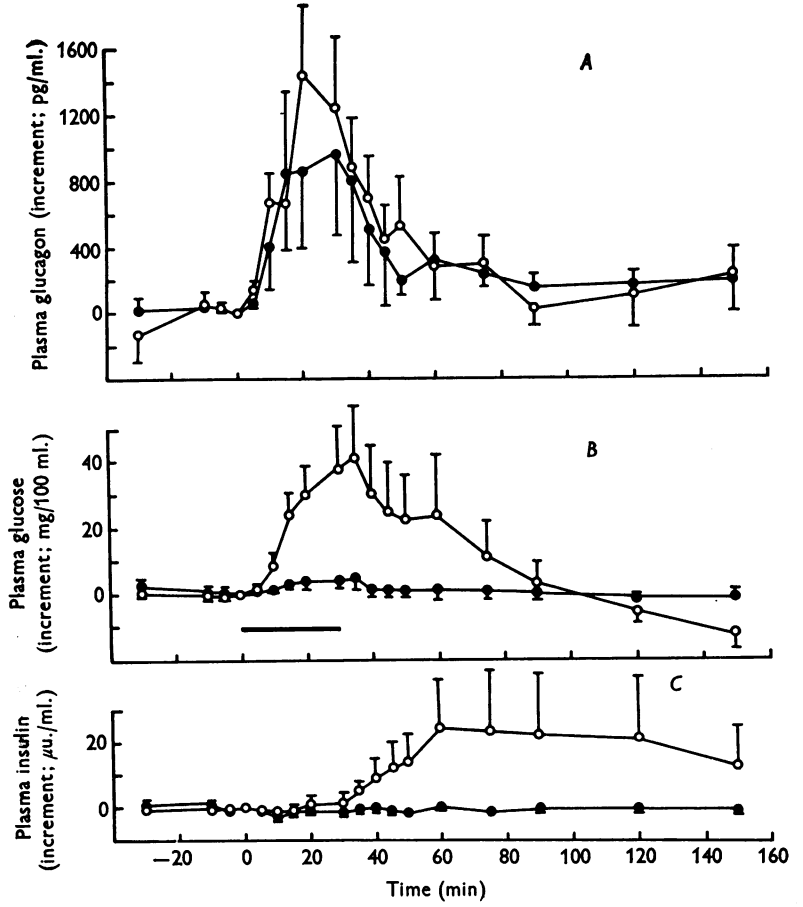

Fig. 9. Comparison of the changes in mean plasma glucagon, glucose and insulin concentration in groups of 3–5 week old calves in response to grade 1 (○; n = 6) or grade 2 (●; n = 5) hypercapnia. Vertical bars: S.E. of each mean value. Horizontal bar: duration of stimulus.

Plasma glucagon concentration rose abruptly in response to both grade 1 and 2 hypercapnia and, although slightly higher peak values were recorded during grade 1 hypercapnia (1440 ± 430 pg/ml.) than during

grade 2 hypercapnia (970 ± 490 pg/ml.), the pattern of the two responses was otherwise similar (Fig. 9). The extent of the rise in plasma glucagon concentration during grade 1 hypercapnia is comparable with that

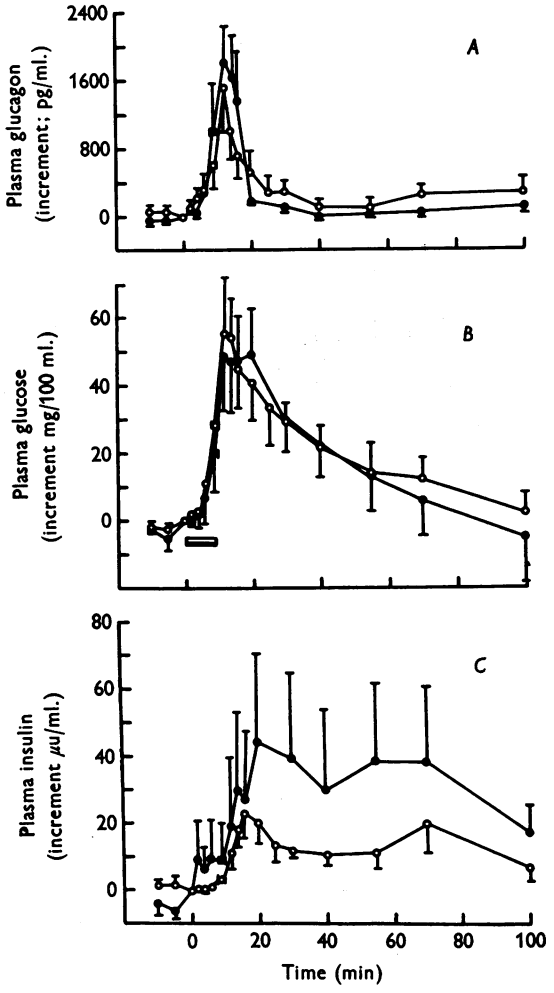

Fig. 10. Comparison of the changes in mean plasma glucagon, glucose and insulin concentration in 3–5 week old calves in response to intense hypoxia. ○, normal controls (n = 6; from Bloom *et al.* 1976), ●, calves with cut splanchnic nerves given atropine (0·2 mg/kg; n = 4). Vertical bars: S.E. of each mean value. Horizontal bar: duration of stimulus.

which occurs in response to intense hypoxia (1554 ± 531 pg/ml.; Fig. 10) and is considerably greater than that described above for grade 1 hypoxia. The rise in plasma glucagon concentration during grade 1 hypercapnia

was followed by a steady rise in plasma insulin concentration over the next 30 min, presumably in response to the existing hyperglycaemia. No significant change in either plasma glucose or insulin concentration occurred in response to grade 2 hypercapnia.

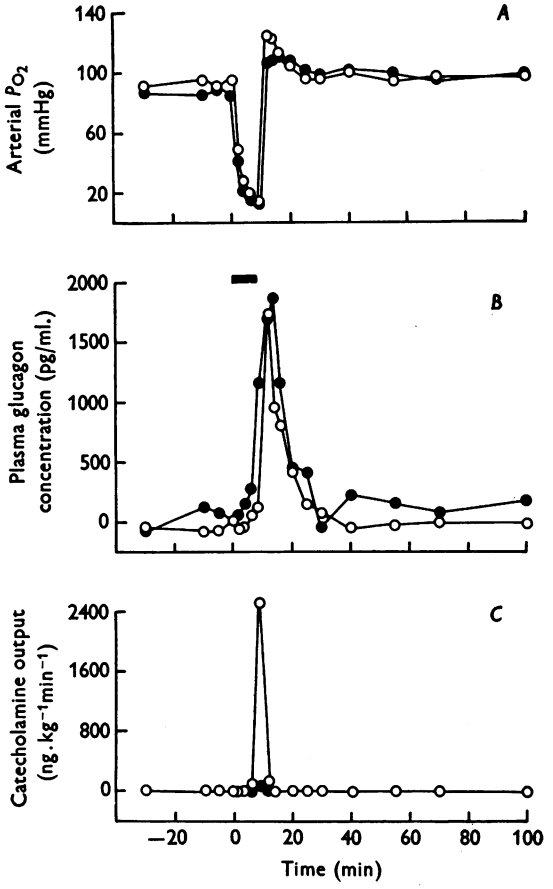

Fig. 11. Comparison of the changes in arterial P_{O_2}, plasma glucagon concentration and catecholamine output from the right adrenal gland in two 3–5 week old calves in response to intense hypoxia. ○, normal control; ●, cut splanchnic nerves. Horizontal bar: duration of stimulus.

Pancreatic responses to hypoxia in calves with cut splanchnic nerves given atropine

The mechanism underlying the release of pancreatic glucagon in response to hypoxia was investigated in 3–5 week old calves in which both splanchnic nerves had been cut, given atropine at a dose of 0·2 mg/kg. Autonomic blockade was found to have no effect on the changes in plasma

glucagon or glucose concentration which occurred in response to intense hypoxia, induced by infusing nitrogen alone through the 'helmet' at a rate of 15 l./min for 8–10 min (Fig. 10*A*, *B*). Comparison of the responses of atropinized calves, in which both splanchnic nerves had been cut, with those of normal animals exposed to the same stimulus shows that the rates of rise of plasma glucagon and glucose are closely similar, as are the extents of the two responses and the rates of recovery after withdrawal

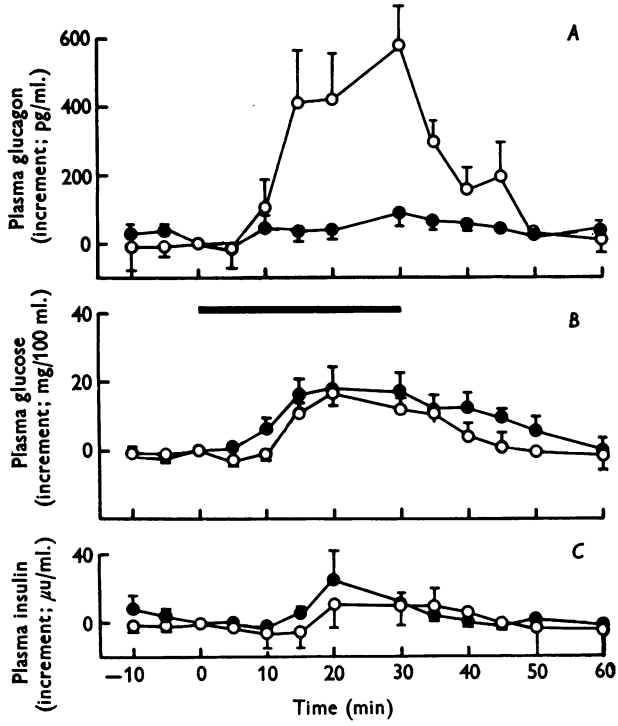

Fig. 12. Comparison of the changes in mean plasma glucagon, glucose and insulin concentration in 3–5 week old calves in response to grade 1 hypoxia. ○, control animals (n = 7); ●, animals with cut splanchnic nerves given atropine (0·1 mg/kg; n = 5). Vertical bars: S.E. of each mean value. Horizontal bar: duration of stimulus.

of the stimulus. Higher mean plasma insulin concentrations were recorded in the experimental group, but there was also more variation between individuals (Fig. 10*C*).

Release of catecholamines from the adrenal medulla is critically dependent on the intensity of the hypoxic stimulus and only occurs at very low arterial oxygen tensions. For this reason, the responses of control calves to intense hypoxia have been matched with those of animals with cut

splanchnic nerves given atropine. Comparison of results from animals in which the changes in arterial P_{O_2} were closely similar (as in Fig. 11) shows that autonomic blockade effectively abolishes the release of catecholamines from the adrenal medulla without significantly reducing the rate of release of pancreatic glucagon.

In contrast, autonomic blockade by section of the splanchnic nerves and administration of atropine effectively abolished release of pancreatic glucagon in response to grade 1 hypoxia (Fig. 12*A*) without significant alteration to the changes in plasma glucose or insulin concentration (Fig. 12*B*, *C*). It is therefore concluded that hypoxia acts directly on the pancreatic cell to stimulate glucagon release if the stimulus is sufficiently intense (arterial P_{O_2} below 20 mmHg). At slightly higher O_2 tensions, release of pancreatic glucagon in response to hypoxia is dependent upon the integrity of the autonomic innervation.

DISCUSSION

These results provide direct evidence that release of adrenal glucocorticoids occurs extremely rapidly in response to either hypoxia or hypercapnia in the conscious calf and are in accord with observations in conscious animals of other species (Marks *et al.* 1965; Lau, 1971*b*; Boddy *et al.* 1974). In both the sheep (Boddy *et al.* 1974) and the rat (Marks *et al.* 1965) hypoxia causes a rise in plasma corticotrophin, concentration and all the evidence from the present study indicates that the adrenal cortical response to either of these respiratory stresses is mediated by release of endogenous corticotrophin. The maximal rates of release of the adrenal glucocorticoids in response to either stimulus fell within the range previously shown to be maximal in response to exogenous corticotrophin (Edwards *et al.* 1975). The ratio of cortisol:corticosterone released from the gland in response to either intense hypoxia or hypercapnia (grade 1) fell to virtually the same value as that which obtains during infusions of maximal doses of corticotrophin. Furthermore, adrenal blood flow rose with increase in steroid output as it does in response to corticotrophin. It therefore seems reasonable to suppose that the adrenal cortical response depends upon hypothalamic activity consequent upon effects of a low P_{O_2} or high P_{CO_2} monitored either centrally or by peripheral chemoreceptors or both.

Section of both splanchnic nerves very substantially reduced the adrenal medullary response to intense hypoxia and it is at least possible that the small amounts of adrenaline and noradrenaline that were released could have been accounted for by failure to denervate the gland completely. This chromaffin tissue evidently has little, if any, capacity

to respond directly to hypoxia in the conscious calf at this age, as has been shown by Comline & Silver (1966*a*). It follows that the adrenal medullary response resembles that of the adrenal cortex in that both are mediated via the central nervous system. However, the disparity between the relative sensitivities of the two responses suggests that there are substantial differences between the respective central mechanisms.

The results of the present experiments provide strong confirmation of the relative resistance of the adrenal medulla to hypoxia and to hypercapnia in the conscious animal, in good agreement with the findings of others who have studied anaesthetized animals (von Euler & Folkow, 1953; Celander, 1954; Tenney, 1956; Miller, 1960; Comline & Silver, 1961). In several other respects they emphasize the need for caution in extrapolating results obtained *in vitro*, or under anaesthesia, to the conscious animal. First, it has been suggested that glucagon may act, at least in part, by stimulating release of adrenaline from the adrenal medulla (Scian, Westerman, Verdesca & Hilton, 1960; Sarcione, Back, Sokal, Mehlman & Knoblock, 1963). In the present experiments, intense hypoxia produced a pronounced increase in plasma glucagon concentration in calves with cut splanchnic nerves, yet only trivial amounts of adrenaline were released from the adrenal medulla. Similarly, grade 2 hypercapnia caused substantial release of pancreatic glucagon without significantly changing either adrenaline or noradrenaline release. Secondly, it has been found that chemoreceptor stimulation causes release of catecholamines from the adrenal medulla in anaesthetized cats and dogs (Critchley, Ungar & Welburn, 1973). The amounts of adrenaline and noradrenaline released during these experiments were extremely small (< 10 ng kg^{-1} min^{-1}) and well below the rate necessary to produce any peripheral effect. However, these results have been advanced in support of the claim that there is a reflex release of adrenal medullary amines in response to chemoreceptor stimulation (Critchley & Ungar, 1974) and that it may be mediated via the pituitary-adrenal cortical axis (Critchley, Henderson, Moffat, Ungar, Waite & West, 1976). The results of the present experiments show that, if there is any such reflex release of catecholamines in the conscious calf, it is remarkably resistant to stimulation, either by low P_{O_2} or high P_{CO_2}. The fact that catecholamine output was either very low or non-existent, while glucocorticoid output was near-maximal, during either hypoxia (grade 1) or hypercapnia (grade 2) shows that glucocorticoids *per se* exert no important effect on catecholamine release in the conscious calf at this age, although they may well influence the rates at which the two amines are synthesized (see for instance Weiner, 1975).

The output of adrenaline far exceeds that of noradrenaline when adrenal catecholamine secretion rises to physiologically effective levels in

response to intense hypoxia in the conscious calf (Bloom *et al.* 1976). In contrast, roughly twice as much noradrenaline as adrenaline is released from the adrenal medulla in the anaesthetized calf at the same age (3 weeks) in response to intense asphyxia (Comline & Silver, 1966*a*). In the foetal lamb the adrenal medullary response can be attributed entirely to the fall in arterial P_{O_2} and the response is not modified by coexistent hypercapnia or acidosis (Comline & Silver, 1966*b*). The present finding that intense hypercapnia causes the release of small but significant amounts of adrenal catecholamines, and predominantly of noradrenaline, indicates that the calf differs from the foetal lamb in this respect. This particular point was examined in four 3–5 week old calves in which asphyxia was induced using a gas mixture of either 5 or 10% CO_2 in N_2, to which the animals were exposed for 8–10 min. The results of these preliminary experiments suggest that hypercapnia potentiates the adrenal medullary response to hypoxia in the conscious calf during asphyxia. Higher mean rates of output of both adrenaline (asphyxia = 923 ± 279 ng kg^{-1} min^{-1}; hypoxia = 581 ± 349 ng kg^{-1} min^{-1}) and noradrenaline (asphyxia = 925 ± 154 ng kg^{-1} min^{-1}; hypoxia = 332 ± 200 ng kg^{-1} min^{-1}) were achieved during asphyxia together with proportionately higher amounts of noradrenaline. In anaesthetized calves of the same age catecholamine output from a single adrenal gland during asphyxia was found to vary with the type of anaesthetic as follows. Under chloralose anaesthesia, noradrenaline output (*ca.* 30 μg/min $\equiv$ 1000 ng.kg^{-1} min^{-1}, assuming a body weight of *ca.* 30 kg) was closely similar to that in the conscious calf, but about twice as great as the adrenaline output (*ca.* 15 μg/min $\equiv$ *ca.* 500 ng.kg^{-1} min^{-1}) (Comline & Silver, 1966*a*). The outputs of both amines were considerably reduced in animals given pentobarbitone (noradrenaline = *ca.* 12 μg/min; adrenaline = *ca.* 3 μg/min). It therefore appears that, whereas barbiturate anaesthesia substantially depresses the output of both amines in response to asphyxia, chloralose reduces the output of adrenaline without significantly altering the rate at which noradrenaline is released from the gland.

The changes in plasma insulin concentration which occurred in response to either hypoxia or hypercapnia were consistent with the general conclusion that release of pancreatic insulin was invariably secondary to hyperglycaemia. During grade 1 hypercapnia this response was delayed until after the stimulus was withdrawn. The output of adrenal catecholamines was too low to account for the rise in plasma glucose concentration that occurred during these experiments. It is therefore likely that this hyperglycaemic response was due to increased activity of the sympathetic innervation to the liver, stimulating glycogenolysis directly (Edwards & Silver, 1970), and the pancreas, promoting release of glucagon (Bloom,

Edwards & Vaughan, 1973). Increased sympathetic activity could also account for the delay in the secretion of insulin as stimulation of the sympathetic innervation to the pancreas, at quite low frequencies, strongly inhibits the release of insulin (Bloom *et al.* 1973).

Both grade 1 and grade 2 hypercapnia proved effective stimuli to secretion of pancreatic glucagon, whereas no secretion of adrenal catecholamines occurred during grade 2 hypercapnia. Evidently the sensitivity of the pancreatic α cell to hypercapnia is greater than that of the adrenal medulla. The sensitivity of the pancreatic glucagon release mechanism to hypoxia was found to be intermediate between that of the adrenal cortex on the one hand and the adrenal medulla on the other. At a mean arterial P_{O_2} of $20{\cdot}3 \pm 1{\cdot}8$ mmHg (grade 1 hypoxia) a substantial rise in plasma glucagon concentration occurred (Fig. 9) while comparatively trivial amounts of adrenaline and noradrenaline were released from the adrenal medulla (Fig. 4). This pancreatic endocrine response was abolished by autonomic blockade. It therefore appears that, at least in the calf, the adrenal medulla is less responsive to the effects of hypoxia mediated via the innervation than are the α cells of the pancreatic islets.

The work has been supported by grants from the Agricultural Research Council, the British Diabetic Association, the Medical Research Council and the Wellcome Trustees. We are also indebted to Dr D. M. Burley (Ciba) for his continued support. It is a particular pleasure to acknowledge the skilled technical assistance of Mrs K. P. Lindsell, Miss A. West and Mr P. M. M. Bircham.

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