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ADRENAL MEDULLARY RESPONSES TO SPLANCHNIC NERVE STIMULATION IN NEW-BORN CALVES

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SUMMARY

1. Right adrenal medullary and various cardiovascular responses to stimulation of the peripheral end of the right splanchnic nerve have been investigated in conscious calves 24–48 h after birth and compared with those obtained previously in older conscious calves.

2. The results show that splanchnic nerve stimulation produces a smaller rise in mean aortic blood pressure and haematocrit in new-born calves than occurs in older animals. The fall in adrenal vascular resistance is also reduced, as is the amount of catecholamine released from the adrenal medulla, at 24–48 h.

3. Although the absolute amounts of adrenaline and noradrenaline released in response to splanchnic nerve stimulation were significantly less in 24–48 h old calves, the ratio (3:2) was the same as that found in 2–5 week old animals and that in which the two amines are stored in the gland.

4. The results confirm the contention that the sympathetic innervation is relatively immature immediately after birth in this species and that functional maturation occurs gradually over a period of weeks. However, they also show that the ratio in which adrenaline and noradrenaline are released from the adrenal medullae provide no index of this process, as had previously been suggested.

INTRODUCTION

Studies in anaesthetized calves originally suggested that the sympathetic system is relatively immature at birth in this species and that this immaturity is reflected in a gradual change in the ratio of noradrenaline: adrenaline that is released from the adrenal medullae over the first few weeks after birth (Silver, 1960; Comline & Silver, 1966). More recent experiments in conscious calves have shown that the output of adrenaline exceeds that of noradrenaline in 2–5 week old calves, during stimulation of the peripheral end of the splanchnic nerve, whereas the reverse situation obtains under anaesthesia at this age (reviewed by Silver & Edwards, 1980).

The finding that anaesthetic agents distort adrenal medullary responses to splanchnic nerve stimulation in 2–5 week old calves does not eliminate the possibility that functional development of the gland is reflected by predominant release of noradrenaline in younger animals. This question has been addressed in the present investigation by examining the adrenal medullary responses of much younger calves

(24–48 h after birth) to splanchnic nerve stimulation, and employing the 'adrenal-clamp' technique in order to do so in unanaesthetized animals. The results confirm that the sympathetic system is relatively immature in a number of respects immediately after birth in these animals. In the case of the adrenal medulla this is reflected in a reduction in the absolute amounts of the two amines released but not in the noradrenaline:adrenaline ratio.

METHODS

Animals

Pedigree Jersey calves were obtained from local farms within 24 h after birth and the experiments then carried out 24 h later to allow sufficient time for preparatory surgery and complete recovery therefrom.

Experimental procedures

Anaesthetic, surgical, post-mortem and experimental procedures were closely similar to those described in detail previously (Edwards, Hardy & Malinowska, 1974; Edwards, Furness & Helle, 1980). Briefly, preparatory surgery was carried out under halothane anaesthesia and involved removal of the right kidney followed by implantation of a specially designed clamp to permit collection of the whole of the effluent blood from the right adrenal gland periodically when required. Narrow-bore polyethylene catheters were inserted into the saphenous arteries so that the tips lay in the abdominal aorta. These catheters were used subsequently to monitor aortic blood pressure and for collection of aortic blood samples. The right splanchnic nerve was cut immediately below the diaphragm and the peripheral end enclosed in a fluid electrode constructed of silicone rubber and silver wire.

In each experiment a standard 10–20 V square-wave stimulus (pulse width 1 ms) was delivered at a continuous frequency of either 4 or 10 Hz for 10 min and was invariably below behavioural threshold. Heart rate and aortic blood pressure were monitored continuously, by means of a Devices L 221 pressure transducer connected to a Devices M19 recorder. Right adrenal blood flow was estimated gravimetrically and corrected for haematocrit percentage before the output of catecholamines from the gland was estimated. Adrenal vascular resistance was estimated by dividing the perfusion pressure (aortic blood pressure) by the adrenal blood flow at the time.

Analytical procedures

Arterial blood samples were collected into heparinized tubes for haematocrit estimations. Adrenal venous effluent blood was collected into heparinized tubes containing EDTA for catecholamine estimations and centrifuged as soon as possible at +4 °C; the plasma was then sequestered at −20 °C. Adrenaline and noradrenaline were measured by a modification of Euler and Floding's trihydroxyindole method (Euler & Floding, 1955) as described previously (Bloom, Edwards, Hardy, Malinowska & Silver, 1975).

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

RESULTS

Release of adrenal catecholamines in response to splanchnic nerve stimulation

Continuous stimulation of the peripheral end of the right splanchnic nerve at 4 Hz for 10 min produced an increase in the output of both noradrenaline and adrenaline from the right adrenal gland in a group of four conscious calves 24–48 h after birth. Enhanced release of both amines persisted throughout the period of stimulation and the output of both subsided rapidly to the normal range when the stimulus was discontinued (Fig. 1). The average mean output of adrenaline during stimulation (48 ± 1 ng kg^{-1} min^{-1}) was significantly greater than that of noradrenaline

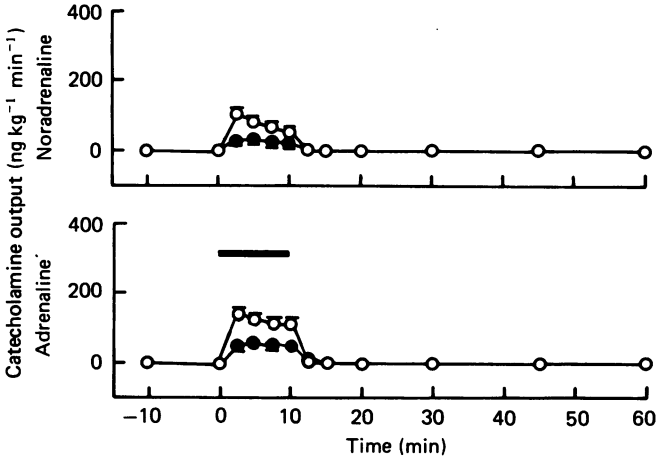

Fig. 1. Comparison of the output of catecholamines from the right adrenal medulla in response to stimulation of the peripheral end of the right splanchnic nerve at 4 Hz continuously for 10 min in conscious 24 h old calves (●; $n = 4$) with that of 2–5 week old calves (○; $n = 6$; from Edwards *et al.* 1980). Vertical bars: S.E. of each mean value where these exceed the size of the symbol. Horizontal bar: duration of stimulus.

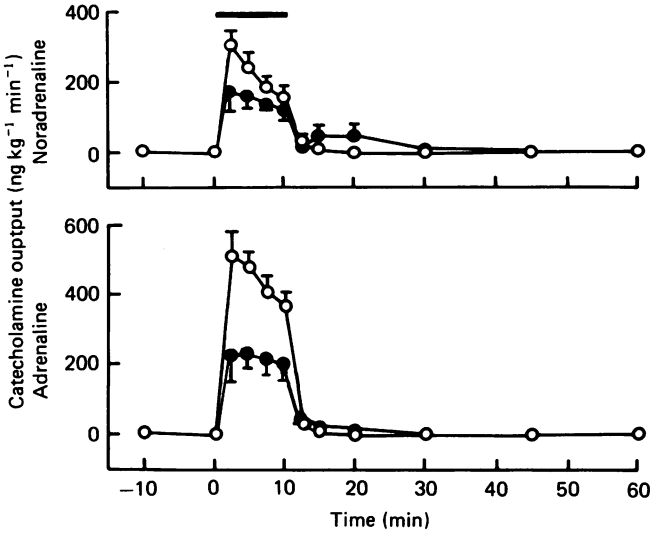

Fig. 2. Comparison of the output of catecholamines from the right adrenal medulla in response to stimulation of the peripheral end of the right splanchnic nerve at 10 Hz continuously for 10 min in conscious 24–48 h old calves (●; $n = 4$) with that of 2–5 week old calves (○; $n = 6$; from Edwards *et al.* 1980). Vertical bars: S.E. of each mean value where these exceed the size of the symbol. Horizontal bar: duration of stimulus.

(29 ± 3 ng kg^{-1} min^{-1}; $P < 0{\cdot}01$) exceeding it in the approximate ratio 3:2, just as in older conscious calves (Edwards *et al.* 1980). However, the amounts of both amines that were released were significantly less than those in the older animals. Thus, the peak output of noradrenaline in 2–5 week old calves (105 ± 16 ng kg^{-1} min^{-1}) was

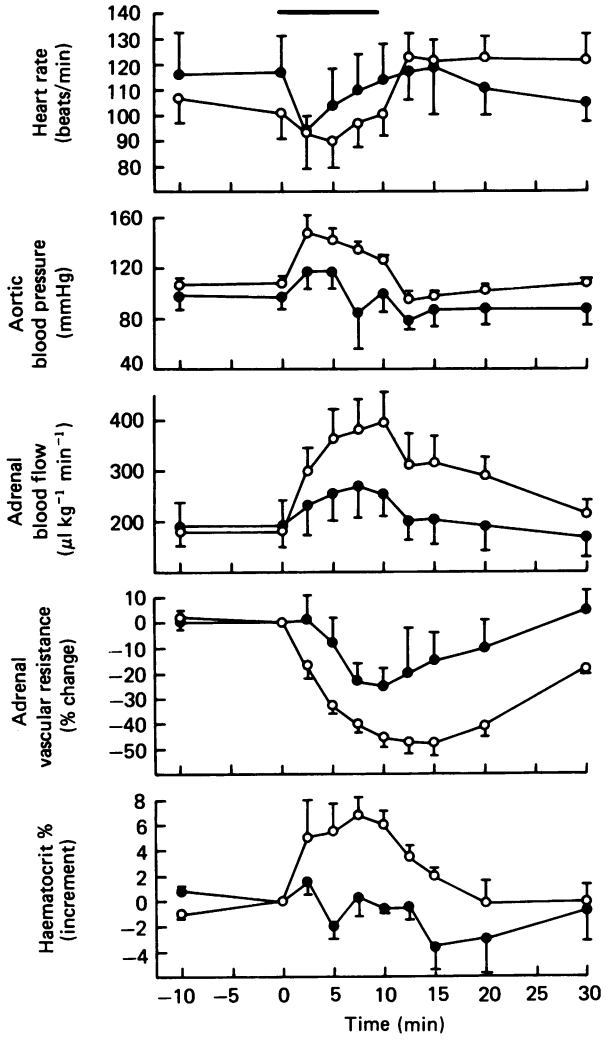

Fig. 3. Comparison of various cardiovascular responses to stimulation of the peripheral end of the right splanchnic nerve at 4 Hz continuously for 10 min in conscious 24–48 h old calves (●; $n = 4$) with those of 2–5 week old calves (○; $n = 6$; from Edwards *et al.* 1980). Vertical bars: S.E. of each mean value. Horizontal bar: duration of stimulus.

about three times greater than that achieved in the new-born group (34 ± 10 ng kg^{-1} min^{-1}; $P < 0{\cdot}01$), as was that of adrenaline (142 ± 13 at 2–5 weeks compared with 50 ± 7 at 24–48 h; $P < 0{\cdot}01$; Fig. 1).

Continuous stimulation of the peripheral end of the right splanchnic nerve at 10 Hz for 10 min produced an abrupt and more substantial increase in the output of both

catecholamines, which was also maintained for the duration of the stimulus (showing less tendency to decline with the passage of time than the corresponding responses in older animals) and declined rapidly when the stimulus was discontinued (Fig. 2). The average mean output of adrenaline during stimulation (219 ± 6 ng kg^{-1} min^{-1})

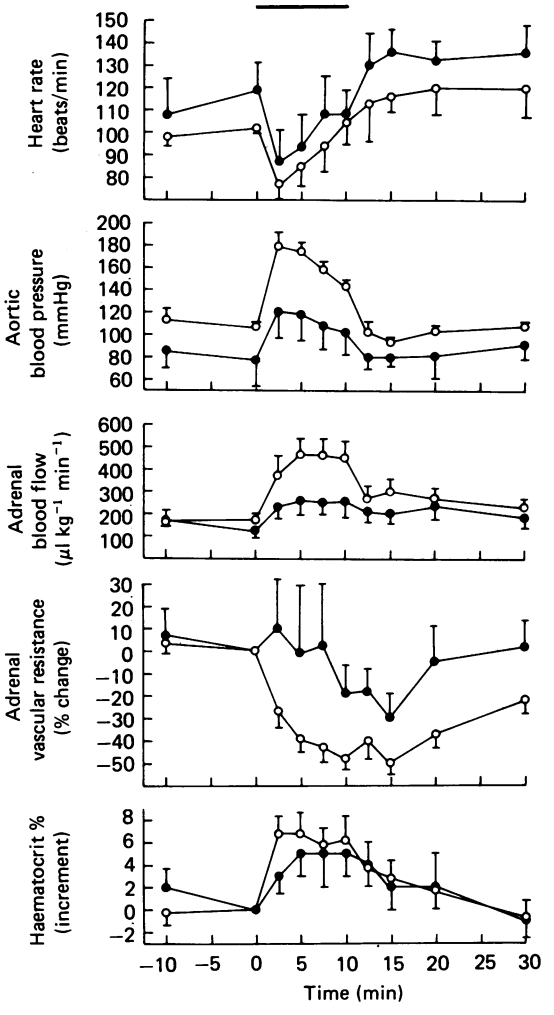

Fig. 4. Comparison of various cardiovascular responses to stimulation of the peripheral end of the right splanchnic nerve at 10 Hz continuously for 10 min in conscious 24–48 h old calves (●; $n = 4$) with those of 2–5 week old calves (○; $n = 6$; from Edwards *et al.* 1980). Vertical bars: S.E. of each mean value. Horizontal bar: duration of stimulus.

was also significantly greater than that of noradrenaline (147 ± 22 ng kg^{-1} min^{-1}; $P < 0{\cdot}02$) and exceeded it in about the same proportion (3:2). As was found with stimulation at 4 Hz greater amounts of both amines were released during stimulation in the older group of animals. The mean peak outputs of noradrenaline were 312 ± 38 ng kg^{-1} min^{-1} at 2–5 weeks and 170 ± 56 at 24–48 h ($P > 0{\cdot}05$; n.s.) and of adrenaline 505 ± 72 at 2–5 weeks and 232 ± 41 at 24 h ($P < 0{\cdot}02$).

Cardiovascular responses to splanchnic nerve stimulation

Comparison of the changes in those cardiovascular parameters that were monitored during these experiments provides further evidence of immaturity of the splanchnic sympathetic innervation at birth in the calf. Stimulation of the peripheral end of the right splanchnic nerve at 4 Hz produced a relatively small and transient rise in mean aortic blood pressure at 24–48 h whereas hypertension was maintained for the duration of the stimulus in the older animals (Fig. 3). This difference in perfusion pressure presumably contributed to the fact that there was also a much more substantial rise in adrenal blood flow in the 2–5 week old group. However, the fact that the changes in blood flow were not entirely due to changes in aortic blood pressure is attested to by the finding that stimulation invariably produced a fall in adrenal vascular resistance and this was also significantly greater in the older animals (Fig. 3). More obvious, albeit circumstantial, evidence of sympathetic inadequacy is provided by comparison of the changes in haematocrit. Stimulation at 4 Hz produced no significant change in the mean haematocrit at 24–48 h whereas it rose to a peak incremental value of $5{\cdot}9 \pm 1{\cdot}2\,\%$ in the 2–5 week old group, equivalent to an increase in circulating red blood cells of about 20 %.

Stimulation at 10 Hz produced a more persistent hypertensive response at 24–48 h but mean aortic pressure was invariably less than that of the older animals before, during and after stimulation. This was reflected in a significantly smaller rise in adrenal blood flow in the former group, associated with a marked delay in adrenal vasodilatation (Fig. 4). In contrast, stimulation at this higher frequency produced a significant rise in haematocrit at 24–48 h which, although it was delayed, had risen by about the same amount as in the older group 7·5 min after stimulation was initiated (Fig. 4).

DISCUSSION

The results of these experiments show that a number of responses to stimulation of the peripheral end of the splanchnic nerve are significantly reduced during the first 2 days after birth in the calf, even when the experiments are carried out in conscious animals in order to avoid the depressant effects of anaesthetic agents. Such responses include the rise in mean aortic blood pressure which presumably reflects an increase in peripheral resistance as it is accompanied by pronounced bradycardia, the rise in haematocrit, the fall in adrenal vascular resistance and reduced output of catecholamines from the adrenal medulla. This list can be extended to include heptatic glycogenolysis and release of pancreatic glucagon, both of which have been shown to be significantly reduced in anaesthetized calves of the same age (Edwards & Silver, 1970; Bloom, Edwards & Vaughan, 1973).

The precise cause of this apparent immaturity of sympathetic mechanisms at birth is not clear and could be due to deficiency of either the innervation or the particular effector or both. In the case of the liver there is evidence that hepatic phosphorylase is activated less readily in response to both adrenaline and glucagon and both these hormones elicit relatively poor hyperglycaemic responses in new-born animals; this together with other evidence relating to the development of the sympatho-adrenal system has been the subject of an extensive review (Silver & Edwards, 1980). In 2–5

week old anaesthetized adrenalectomized calves an apparently maximal increase in haematocrit occurs in response to continuous stimulation of the peripheral ends of the splanchnic nerves at 1 Hz (Edwards, 1972). Assuming that this response depends, at least in part, on splenic contraction the observation is in accordance with Celander's classical study in the cat, which showed that almost maximal changes in splenic surface area occurred at much lower frequencies than those needed to elicit maximal sympathetic vasoconstriction (Celander, 1954). In the present experiments the rise in mean haematocrit in response to stimulation at 4 Hz was significantly less in 24–48 h calves than in older animals, but was substantially enhanced by increasing the stimulus frequency to 10 Hz. Thus, the effector cells are capable of producing a comparable maximum response in both groups but a much higher frequency is needed in the younger animals, which suggests that the primary deficiency is in development of the innervation or of a sufficiency of receptor sites in this instance. The same explanation could account for the difference which was observed in the hypertensive responses of the two groups.

Immaturity of the adrenal gland at 24–48 h was reflected both by a significant reduction in the vasodilator response and in the absolute amounts of adrenaline and noradrenaline that were released. The cause of the fall in adrenal vascular resistance which normally occurs during splanchnic nerve stimulation in these animals has yet to be established. Adrenocorticotrophin is known to be a potent vasodilator agent (Edwards, Hardy & Malinowska, 1975) but it is unlikely that the reduction in this response in the new-born animals in the present study was attributable to immaturity of the pituitary–adrenal axis as comparison of the responses of conscious calves of the same ages to 2-deoxyglucose has shown that there is a closely similar rise in mean plasma cortisol concentration (Bloom & Edwards, 1981). The recent finding that vasoactive intestinal peptide, which is an extremely potent vasodilator agent, is present in the gland and released in response to splanchnic nerve stimulation, at least in older calves (S. R. Bloom & A. V. Edwards, unpublished observations) provides a clue for future study but insufficient grounds for further speculation at this stage.

The finding that the immaturity of the adrenal medulla, during the first few days after birth, is manifested solely by a reduction in the absolute amounts of adrenaline and noradrenaline that are released in response to stimulation of the splanchnic innervation and *not* by any change in the ratio, as had previously been supposed, is in precise accord with other studies of adrenal medullary function in the conscious calf. Thus in 2–5 week old calves the output of adrenaline invariably exceeds that of noradrenaline in response to hypoglycaemia (Bloom *et al.* 1975) and hypoxia (Bloom, Edwards & Hardy, 1977). In 24–48 h old calves the brain is protected from the effects of prolonged and severe insulin hypoglycaemia by the release of adrenaline from the adrenal medulla. This protective mechanism is abolished by prior section of both splanchnic nerves and can be mimicked by an I.V. infusion of adrenaline (but not noradrenaline) at a dose of 165 ng kg^{-1} min^{-1} (Comline & Edwards, 1968) which is well below the mean output from a single gland in response to splanchnic nerve stimulation at 10 Hz in unanaesthetized calves (see Fig. 2). Furthermore, the ratio (3:2) in which the two amines are released during splanchnic nerve stimulation in conscious calves of both age groups is comparable with that in which they have been found to be present in the unstimulated gland (Comline & Silver, 1966).

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