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THE ADRENAL CONTRIBUTION TO THE NEUROENDOCRINE RESPONSES TO SPLANCHNIC NERVE STIMULATION IN CONSCIOUS CALVES

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SUMMARY

1. The extent to which the adrenal gland contributes to neuroendocrine responses to electrical stimulation of the peripheral end of the splanchnic nerve has been investigated in conscious calves in which the right nerve was stimulated either at 4 Hz continuously for 10 min or at 40 Hz in 1 s bursts at 10 s intervals for the same period.

2. It was confirmed that the release of neuropeptide Y (NPY) and of gastrin-releasing peptide (GRP) is potentiated by stimulation in bursts at a relatively high frequency and shown that the adrenal gland made a negligible contribution to these responses.

3. There was no detectable change in the concentration of vasoactive intestinal peptide (VIP) in the arterial plasma but the existence of a very small but highly significant rise in the output of VIP from the adrenal provided evidence that it was released within the gland in response to splanchnic nerve stimulation.

4. The concentration of calcitonin gene-related peptide (CGRP) in the arterial and adrenal venous effluent plasma was consistently below the level of detection of the assay.

5. Splanchnic nerve stimulation resulted in an abrupt rise in the output of both free and total met^5-enkephalin-like immunoreactivity from the adrenal gland which was substantially potentiated by stimulating in bursts. This pattern of stimulation also increased the proportion released in a high-molecular-weight form.

6. Stimulation in bursts significantly enhanced the output of both adrenaline and noradrenaline from the adrenal and resulted in the release of proportionately more noradrenaline. Small amounts of dopamine and DOPAC were also released during splanchnic nerve stimulation and the output of dopamine was significantly increased by stimulating in bursts.

7. Both patterns of stimulation elicited an abrupt rise in mean plasma adrenocorticotrophic hormone (ACTH) concentration, which was associated with an increase in mean adrenal cortisol output and the former effect was significantly enhanced by stimulating in bursts.

8. It is concluded that certain responses to splanchnic nerve stimulation are significantly potentiated by an intermittent high-frequency pattern of stimulation, including all those that are attributable to adrenal medullary activity, whereas others are apparently unaffected by changes in stimulus pattern.

INTRODUCTION

Previous studies have shown that stimulation of the peripheral ends of the splanchnic nerves in conscious adrenalectomized calves produces an abrupt rise in the concentration of the mammalian form of bombesin (gastrin-releasing peptide, GRP) in the arterial plasma and that this effect is greatly potentiated by intermittent stimulation at a relatively high frequency (Bloom, Edwards & Ghatei, 1984). Release of neuropeptide Y (NPY), which is known to be present in high concentrations in the mammalian adrenal medulla (Allen, Adrian, Polak & Bloom, 1983), is potentiated by intermittent high-frequency stimulation in precisely the same way (Allen, Bircham, Bloom & Edwards, 1984) but it is not known whether an adrenal output makes a significant contribution to either of these responses. It has recently been suggested that yet another peptide, vasoactive intestinal peptide (VIP), which is present in nerve terminals in the adrenal gland (Hökfelt, Lundberg, Schultzberg & Fahrenkrug, 1981; Holzwarth, 1984) might possibly mediate the release of cortisol which occurs in response to splanchnic nerve stimulation in the presence of adrenocorticotrophic hormone (ACTH) (Edwards & Jones, 1987; Bloom, Edwards & Jones, 1987*b*). This contention would acquire greater credibility if it were shown that VIP is actually released from adrenal nerve terminals when the splanchnic nerve is stimulated.

Accordingly, the present study was undertaken to assess the output of these peptides from the adrenal gland and to quantify the outputs of various catecholamines and peptides. This was accomplished by comparing the effect of stimulating the peripheral end of the right splanchnic nerve at either 4 Hz continuously for 10 min or at 40 Hz for 1 s at 10 s intervals, for the same period, in conscious calves, The adrenal clamp technique (Edwards, Hardy & Malinowska, 1974) was employed to collect samples of adrenal venous effluent blood.

METHODS

Animals

Pedigree Jersey calves were obtained from local farms shortly after birth and used at ages ranging between 3 weeks and 2 months (23–35 kg body weight). They were kept in individual pens and maintained on a diet of either cow's milk or artificial milk (Easy-mix Volak; Volak Ltd) at a rate of 3–4 l/day. Food was withheld overnight prior to each experiment.

Experimental procedures

Anaesthesia was induced with chloroform (Chloroform SLR, Fisons) and maintained with halothane (May & Baker, *circa* 2% in O_2). Preparatory surgery involved the insertion of narrow-bore polypropylene or polyvinyl catheters into the saphenous arteries so that the tips lay in the abdominal aorta. These were used subsequently to monitor aortic blood pressure and heart rate and for the collection of arterial blood samples. Following removal of the right kidney, the right renal vein was cannulated and an adrenal clamp emplaced (Edwards *et al.* 1974; Edwards, Furness & Helle, 1980). The right splanchnic nerve was cut immediately below the diaphragm and the peripheral end enclosed in a fluid electrode designed to minimize spread of stimulus to surrounding tissues.

The experiments were carried out 3–4 h after surgery and when the animals had recovered from anaesthesia. A standard 20–30 V square-wave stimulus (pulse width 0·5 ms) was employed at a frequency of either 4 Hz continuously for 10 min or at 40 Hz for 1 s at 10 s intervals for the same period. Heart rate and aortic blood pressure were monitored continuously by means of a Devices M19 recorder. Right adrenal blood flow was estimated gravimetrically and corrected for haematocrit percentage before the outputs of catecholamines and peptides from the gland were calculated. These were estimated from the concentration of each in the adrenal effluent plasma and adrenal plasma flow at the time of collection and expressed as unit weight min^{-1} kg body $weight^{-1}$. Adrenal vascular resistance was estimated by dividing the perfusion pressure (mean aortic blood pressure) by the right adrenal blood flow.

Analytical procedures

Samples of arterial and of right adrenal venous effluent blood were collected at intervals into heparinized tubes containing 2–3 mg EDTA for catecholamine estimations, phenylmethylsulphonyl fluoride (final concentration 0·1 mM; Sigma Chemical Co.) for haematocrit, glucose, ACTH and cortisol estimations and aprotinin (Trasylol; Bayer; final concentration 1000 K.I.U. ml^{-1}) for peptide estimations. Each tube was centrifuged as soon as possible at 4 °C and plasma then sequestered at either −20 or −70 °C.

Glucose was measured enzymatically using a Beckman Mark 2 Glucose Analyzer. Adrenaline and noradrenaline, together with certain of their precursors and metabolites, were measured by HPLC (high-pressure liquid chromatography) with electrochemical detection (Arkinstall & Jones, 1985). ACTH, cortisol, and met-enkephalin were measured by radioimmunoassay as described previously, total met-enkephalin immunoreactivity being determined after trypsin and carboxypeptidase B digestion (Jones, Boddy, Robinson & Ratcliffe, 1977; Edwards, Hansell & Jones, 1986; Edwards & Jones, 1987). Calcitonin gene-related peptide (CGRP) was assayed by a rabbit antiserum raised to synthetic rat α-CGRP and an ^{125}I-labelled histidyl CGRP tracer showing no cross-reaction with other mammalian peptides (Kraenzlin, Ch'ng, Mulderry, Ghatei & Bloom, 1985). Gastrin-releasing peptide (GRP) or mammalian bombesin-like immunoreactivity (BLI) was measured with an antiserum (BN103) raised to synthetic (Lys^3)-bombesin analogue, conjugated with glutaraldehyde to bovine serum albumin, in rabbits. This antiserum cross-reacted 96% with porcine GRP and 0·2% with substance P. There was no cross-reaction with any other gastrointestinal or pancreatic peptide tested (Ghatei, 1982). The radiolabel was ^{125}I-(Tyr^4)-bombesin prepared by the chloramine T method and the sensitivity of the assay was less than 4 pmol l^{-1} (95% confidence limits). NPY was assayed using a rabbit anti-NPY antiserum (YN-10) showing 30% cross-reactivity with peptide YY and no significant cross-reaction with pancreatic polypeptide including both human and bovine varieties (Allen, Yeats, Adrian & Bloom, 1984*b*). Vasoactive intestinal peptide (VIP) was measured by radioimmunoassay using a rabbit antiserum which apparently reacted only with whole VIP (V9) and showed no cross-reaction with other peptides, including peptide histidine isoleucine (Mitchell & Bloom, 1978).

Results are expressed as means ± S.E. of mean. Statistical analyses were made according to Snedecor & Cochran (1967) and statistical significance was determined by the Student's *t* test.

RESULTS

Cardiovascular responses

The changes in mean aortic blood pressure, heart rate, right adrenal blood flow and right adrenal vascular resistance which occurred in response to stimulation of the peripheral end of the right splanchnic nerve for 10 min, at either 4 Hz continuously or at 40 Hz for 1 s at 10 s intervals, are illustrated in Fig. 1. Both patterns of stimulation elicited closely similar changes in each of these parameters and it is noteworthy that there was a highly significant fall in right adrenal vascular resistance in response to splanchnic nerve stimulation. This response is suppressed in conscious hypophysectomized calves by an infusion of exogenous ACTH that raises the concentration of ACTH in the plasma to about 1200 pg ml^{-1} and the output of adrenal cortisol to about 550 ng min^{-1} kg^{-1} (half-maximal). The changes in plasma

ACTH and adrenal cortisol output that occurred in the present study (Fig. 2) were much too small to inhibit the fall in adrenal vascular resistance and attest to the absence of significant stress in these experiments.

Haematocrit and arterial plasma glucose concentration were also monitored and the expected increase in both occurred in response to splanchnic nerve stimulation. Neither response was affected significantly by the pattern of the stimulus.

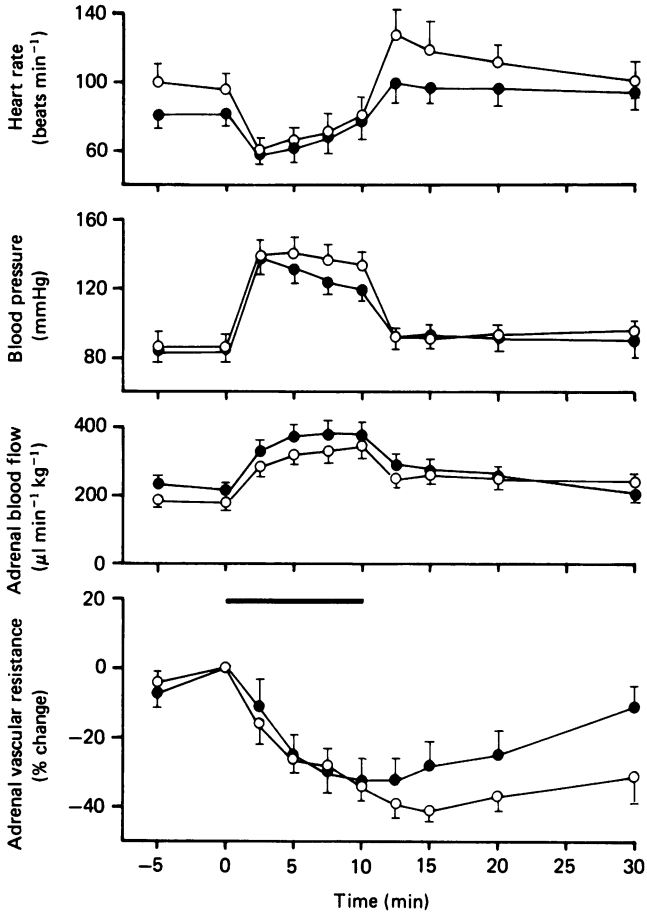

Fig. 1. Comparison of various cardiovascular responses to stimulation of the peripheral end of the right splanchnic nerve for 10 min at either 4 Hz continuously (●) or at 40 Hz for 1 s every 10 s (○) in seven conscious calves. Horizontal bar: duration of stimulus. Vertical bars: S.E. of each mean value.

Release of peptides

Stimulation of the peripheral end of the right splanchnic nerve at 4 Hz for 10 min caused a small but significant rise in mean arterial plasma GRP concentration (by 25 ± 5 pmol l^{-1}) but this effect was greatly enhanced by stimulation at 40 Hz (by 65 ± 14 pmol l^{-1}) at 10 min. The difference between these two peak values was statistically significant ($P < 0.02$). However, the mean concentration of GRP in the right adrenal effluent plasma rose at similar rates to reach closely similar peaks at

10 min (4 Hz: 23 ± 4 pmol l^{-1}; 40 Hz in bursts: 70 ± 21 pmol l^{-1}) showing that release of this peptide from the adrenal gland made no effective contribution to the response. The mean average adrenal GRP output during stimulation at 4 Hz continuously was actually negative ($-0{\cdot}05 \pm 0{\cdot}12$ fmol min^{-1} kg^{-1}) while the corresponding value during stimulation in bursts at 40 Hz ($0{\cdot}73 \pm 0{\cdot}42$ fmol min^{-1} kg^{-1}) did not differ significantly from the mean average value before and after stimulation ($0{\cdot}21 \pm 0{\cdot}09$ fmol min^{-1} kg^{-1}; Fig. 3).

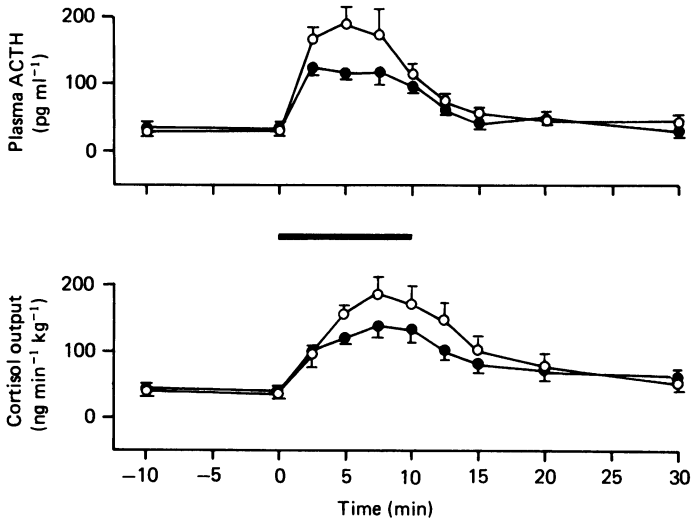

Fig. 2. Comparison of the changes in mean arterial plasma ACTH concentration and mean right adrenal cortisol output in response to stimulation of the peripheral end of the right splanchnic nerve at either 4 Hz continuously (●) or at 40 Hz for 1 s at 10 s intervals (○) in seven conscious calves. Horizontal bar: duration of stimulus. Vertical bars: S.E. of each mean value.

A very similar pattern emerged in the case of NPY where the extent of the rise in the mean concentration in both arterial and adrenal effluent plasma in response to splanchnic nerve stimulation was closely similar and substantially potentiated by stimulating in bursts at the higher frequency (Fig. 4). If the peptide was secreted from the adrenal gland it was in femtomolar amounts, close to the detection limits of the methodology. The mean average adrenal output of NPY during stimulation at 4 Hz continuously was found to be $1{\cdot}5 \pm 0{\cdot}4$ fmol min^{-1} kg^{-1} and significantly exceeded that before and after stimulation ($0{\cdot}5 \pm 0{\cdot}01$ fmol min^{-1} kg^{-1}). However, this was not the case during stimulation at 40 Hz intermittently, in spite of the fact that this latter pattern is so much more effective in releasing this neuropeptide into the circulation generally. The total amount that would have been released during the 10 min period (300 fmol) would not have made a detectable contribution to the observed rise of about 80 pmol l^{-1} in the arterial plasma.

Splanchnic nerve stimulation had no effect on the concentration of VIP in the arterial plasma but very slightly raised that in the adrenal effluent plasma which, together with the enhanced adrenal blood flow, had the effect of significantly increasing the output of the peptide in response to both patterns of stimu-

lation (Fig. 5). Thus the average mean VIP output during stimulation at 4 Hz, $1{\cdot}3 \pm 0{\cdot}2$ fmol min^{-1} kg^{-1}, exceeded the mean average value before and after stimulation ($0{\cdot}3 \pm 0{\cdot}1$ fmol min^{-1} kg^{-1}; $P < 0{\cdot}01$), as did the output during stimulation at 40 Hz in bursts ($0{\cdot}9 \pm 0{\cdot}1$ fmol min^{-1} kg^{-1} compared with $0{\cdot}1 \pm 0{\cdot}1$ fmol min^{-1} kg^{-1}; $P < 0{\cdot}001$).

The concentration of calcitonin gene-related peptide in the arterial and adrenal venous effluent plasma was consistently below the limit of detection of the assay.

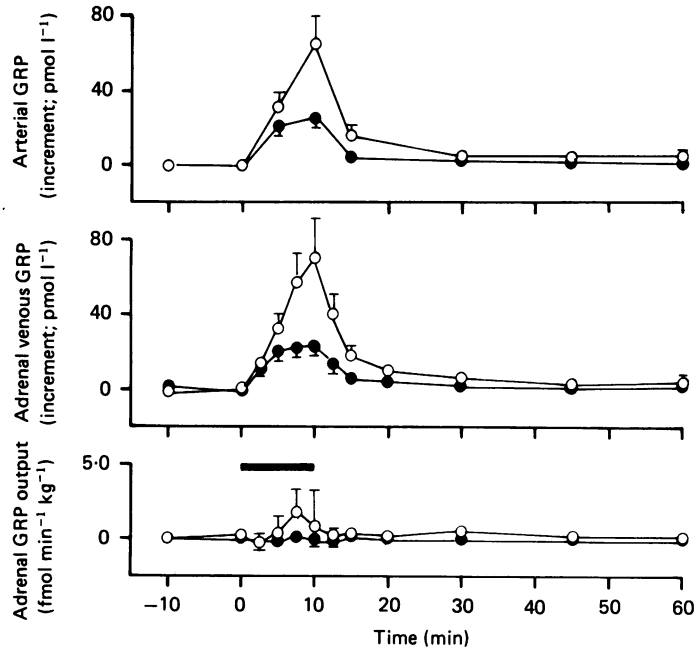

Fig. 3. Comparison of the changes in mean arterial and right adrenal venous plasma GRP concentration, together with right adrenal GRP output, in response to stimulation of the peripheral end of the right splanchnic nerve for 10 min at either 4 Hz continuously (●) or at 40 Hz for 1 s at 10 s intervals (○) in seven conscious calves. Horizontal bar: duration of stimulus. Vertical bars: S.E. of each mean value. Absolute values at time = 0 before continuous stimulation: arterial plasma 10 ± 1 pmol l^{-1}, adrenal venous plasma 10 ± 1 pmol l^{-1}; before stimulation in bursts: arterial plasma 9 ± 1 pmol l^{-1}, adrenal venous plasma 10 ± 1 pmol l^{-1}.

Splanchnic nerve stimulation produced an abrupt rise in the output of enkephalins from the right adrenal gland which was well maintained for the duration of the stimulus (10 min). Stimulation at 4 Hz continuously for 10 min raised the average mean output of free met^5-enkephalin to $3{\cdot}1 \pm 0{\cdot}1$ ng min^{-1} kg^{-1} and that of total met^5-enkephalin to $21{\cdot}8 \pm 1{\cdot}0$ (free:total ratio 1:7). During stimulation at 40 Hz in bursts the corresponding values were $6{\cdot}0 \pm 0{\cdot}4$ (free) and $65{\cdot}0 \pm 5{\cdot}8$ ng min^{-1} kg^{-1} (free:total ratio 1:11). The difference between the two ratios was highly significant ($P < 0{\cdot}001$). Thus the output of both forms of met-enkephalin was substantially increased by stimulating in bursts and a greater proportion of the activity was then released in a high-molecular-weight form (Fig. 6).

Release of catecholamines

Stimulation of the peripheral end of the right splanchnic nerve elicited an abrupt increase in the output of adrenaline and noradrenaline from the gland to plateaux that were well maintained for the duration of the stimulus. During stimulation at 4 Hz continuously the average mean output of noradrenaline

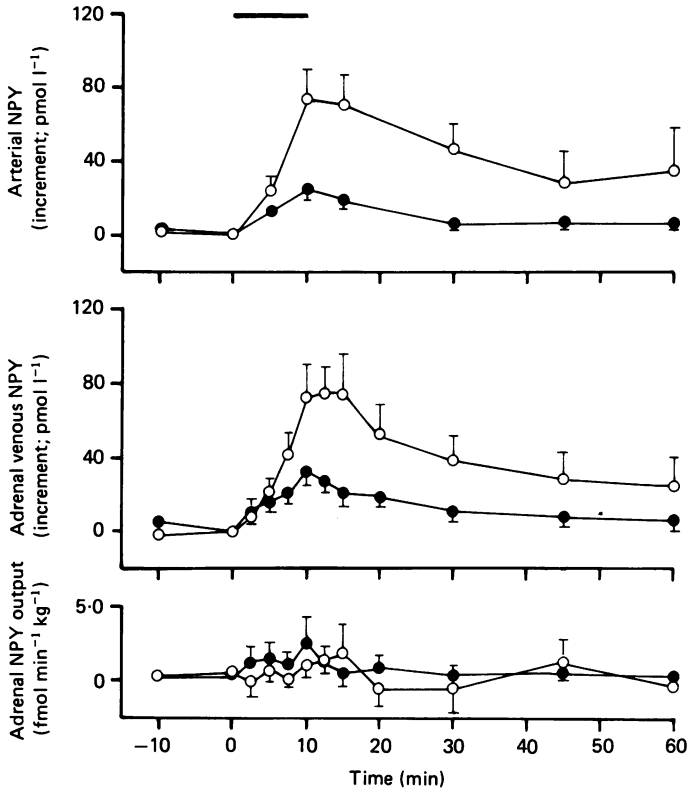

Fig. 4. Comparison of the changes in mean arterial and right adrenal effluent plasma neuropeptide Y concentration, together with mean right adrenal neuropeptide Y output, in response to stimulation of the peripheral end of the right splanchnic nerve for 10 min at either 4 Hz continuously (●) or at 40 Hz for 1 s at 10 s intervals (○) in seven conscious calves. Absolute values at time = 0 before continuous stimulation: arterial plasma 33 ± 4 pmol l^{-1}, adrenal venous plasma 36 ± 5 pmol l^{-1}; before stimulation in bursts: arterial plasma 32 ± 4 pmol l^{-1}, adrenal venous plasma 38 ± 5 pmol l^{-1}.

(169 ± 12 ng min^{-1} kg^{-1}) was similar to that of adrenaline (167 ± 5 ng min^{-1} kg^{-1}) but substantially and significantly greater during stimulation at 40 Hz in bursts (noradrenaline 312 ± 15 ng min^{-1} kg^{-1}; adrenaline 192 ± 3 ng min^{-1} kg^{-1}; $P < 0{\cdot}01$). Thus stimulation in bursts enhanced the output of both amines significantly but led to the release of proportionately moré noradrenaline (Fig. 7).

Small amounts of dopamine and DOPAC were also released in response to splanchnic nerve stimulation and the average mean output of dopamine during

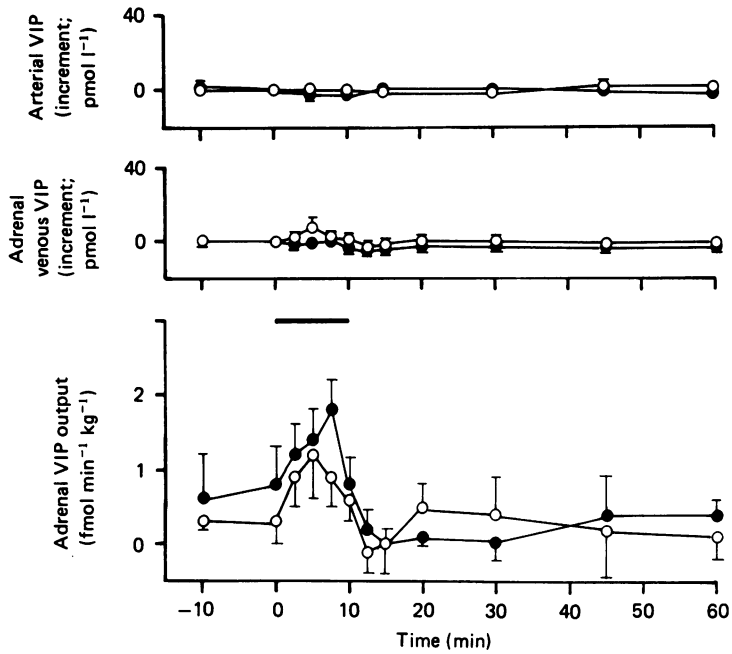

Fig. 5. Comparison of the changes in mean arterial and right adrenal effluent plasma VIP concentration, together with right adrenal VIP output, in response to stimulation of the peripheral end of the right splanchnic nerve for 10 min at either 4 Hz continuously (●) or at 40 Hz for 1 s at 10 s intervals (○) in seven conscious calves. Absolute values at time = 0 before continuous stimulation: arterial plasma 12 ± 2 pmol l^{-1}, adrenal venous plasma 20 ± 3 pmol l^{-1}; before stimulation in bursts: arterial plasma 14 ± 2 pmol l^{-1}, adrenal venous plasma 16 ± 2 pmol l^{-1}.

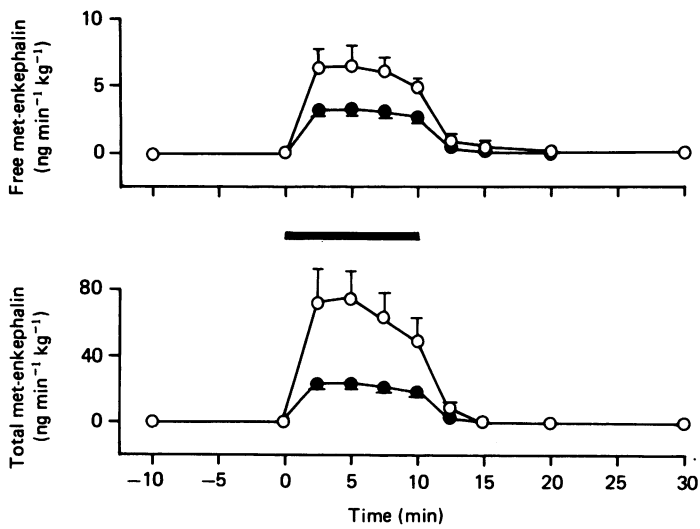

Fig. 6. Comparison of the changes in mean right adrenal free and total met^5-enkephalin output in response to stimulation of the peripheral end of the right splanchnic nerve for 10 min at either 4 Hz continuously (●) or at 40 Hz for 1 s at 10 s intervals (○) in seven conscious calves. Horizontal bar: duration of stimulus. Vertical bars: S.E. of each mean value.

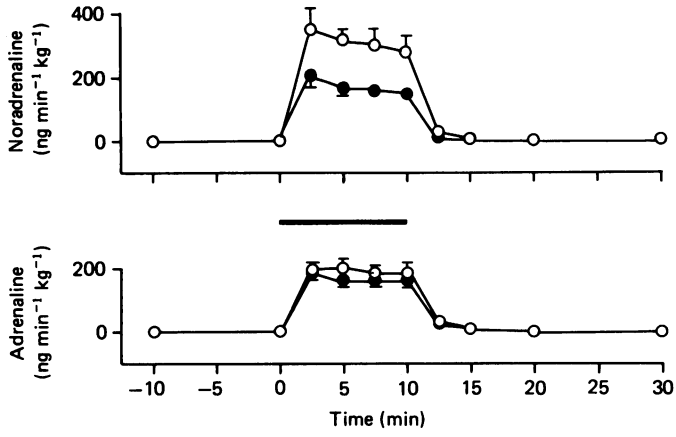

Fig. 7. Comparison of the changes in mean right adrenal adrenaline and noradrenaline output in response to stimulation of the peripheral end of the right splanchnic nerve for 10 min at either 4 Hz continuously (●) or at 40 Hz for 1 s at 10 s intervals (○) in seven conscious calves. Horizontal bar: duration of stimulus. Vertical bars: S.E. of each mean value.

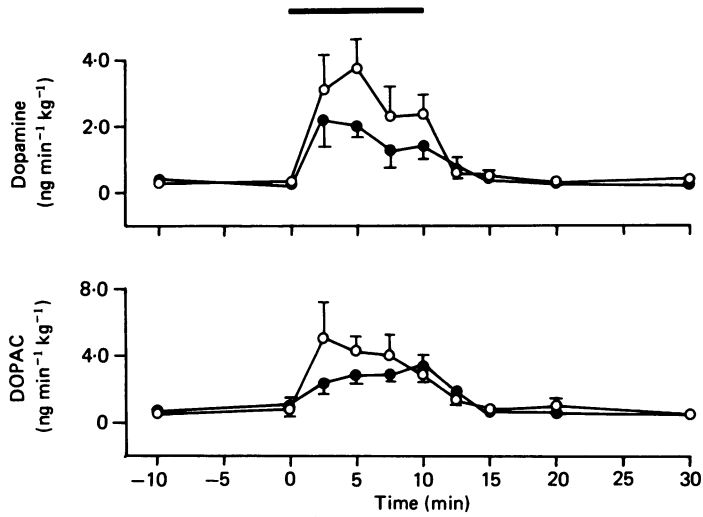

Fig. 8. Comparison of the changes in mean right adrenal dopamine and DOPAC output in response to stimulation of the peripheral end of the right splanchnic nerve at either 4 Hz continuously (●) or at 40 Hz for 1 s at 10 s intervals (○) in seven conscious calves. Horizontal bar: duration of stimulus. Vertical bars: S.E. of each mean value.

stimulation in bursts was significantly greater ($2{\cdot}9 \pm 0{\cdot}3$ ng min^{-1} kg^{-1}) than during continuous stimulation ($1{\cdot}7 \pm 0{\cdot}2$ ng min^{-1} kg^{-1}; $P < 0{\cdot}02$; Fig. 8).

Pituitary and adrenal cortical responses

Both patterns of stimulation elicited an abrupt rise in the concentration of ACTH in the arterial plasma together with a more gradual increase in right adrenal cortisol

output (Fig. 2). Thus the average mean plasma ACTH concentration during continuous stimulation at 4 Hz was 115 ± 6 pg ml^{-1} and that during stimulation in bursts at 40 Hz was 162 ± 16 pg ml^{-1}. The corresponding values for average mean cortisol output were 124 ± 8 and 152 ± 19 ng min^{-1} kg^{-1}. Both these responses were greater during stimulation in bursts than during continuous stimulation but only the difference in ACTH concentration achieved statistical significance ($P < 0.05$).

DISCUSSION

In recent years it has become increasingly apparent that both the adrenal medulla and postganglionic sympathetic nerve terminals contain a range of neuropeptides in addition to acetylcholine and the catecholamines. VIP is present in nerve fibres in the adrenal gland (Linnoila, DiAugustine, Hervonen & Miller, 1980; Hokfelt *et al.* 1981; Holzwarth, 1984) and is released from certain parasympathetic nerve terminals (Bloom & Edwards, 1980). NPY, GRP and enkephalins are present in much higher concentrations in the mammalian adrenal medulla and in postganglionic sympathetic nerve terminals (Linnoila *et al.* 1980; Allen *et al.* 1983; Ekblad, Edvinsson, Wahlestedt, Uddman, Håkanson & Sundler, 1984; de Quidt & Emson, 1986) and are released therefrom by stimulation of the splanchnic nerve (Allen *et al.* 1984*a*; Bloom *et al.* 1984; Edwards *et al.* 1986; Edwards & Jones, 1987). However, the precise source of the neuropeptides that are released into the circulation during stimulation of a sympathetic trunk such as the splanchnic nerve has not been established previously. The present results show that electrical stimulation of the peripheral end of the splanchic nerve in the conscious calf increases the adrenal output of enkephalins substantially and that of VIP modestly, but has no apparent effect on the output of NPY or GRP. The rise in the concentration of these latter two peptides in the peripheral plasma during splanchnic nerve stimulation must therefore have been due to release from postganglionic sympathetic nerve fibres.

This finding is unexpected as it has been shown that quite large increases in the turnover and depletion of adrenal NPY occur in response to insulin hypoglycaemia in rats (de Quidt & Emson, 1986). One would therefore expect that increased splanchnic nerve activity would lead to release of NPY from the adrenal medulla. However, the present results show that any release of NPY from the gland that does occur is trivial by comparison with release from elsewhere. This is particularly surprising in view of the fact that NPY is co-stored with catecholamines in chromaffin granules (Fischer-Coplbrie, Diez-Guerra, Emson & Winkler, 1986) and the two different patterns of stimulation that were employed produced release of adrenaline and noradrenaline in different ratios, indicating activation of quite different populations of these granules. On the other hand, it is in good agreement with the conclusion of Morris and colleagues that only a small proportion of the NPY that is released during severe exercise in man originates from the adrenal glands (Morris, Russell, Kapoor, Cain, Elliot, West, Wing & Chalmers, 1986).

These present results provide a clear illustration of how certain responses to stimulation of a given autonomic nerve pathway are substantially potentiated by an intermittent high-frequency stimulus (up to at least 40 Hz), whereas others are not affected. In this instance we confirmed our previous findings, that cardiovascular

responses to splanchnic nerve stimulation are not affected by the pattern of the stimulus (Bloom *et al.* 1984), whereas release of NPY, GRP and adrenal catecholamines are all substantially potentiated by stimulating in bursts (Edwards, 1982; Allen *et al.* 1984*a*; Bloom *et al.* 1984). It was further established that the same is true for the release of enkephalins, dopamine and cortisol from the adrenal. No pattern has emerged from any of these studies, as yet, which would allow one to predict particular types of response that are likely to be potentiated in this way. Release of neuropeptides from peripheral nerve terminals is often potentiated by stimulating in bursts, as exemplified here by the pattern of release of GRP and NPY from splanchnic postganglionic terminals, and as shown previously with the release of VIP from parasympathetic nerve terminals (Andersson *et al.* 1982). However, responses due exclusively to release of classical transmitters can also be potentiated by stimulating intermittently, as in the case of the secretion of submandibular saliva in response to either parasympathetic or sympathetic stimulation in the cat (C. M. Allen & A. V. Edwards, unpublished observations; Bloom, Edwards & Garrett, 1987*a*) and parotid saliva in the sheep (Andersson *et al.* 1982).

Changing the pattern of stimulation had a profound effect on catecholamine release; stimulation at 40 Hz in bursts resulted in preferential release of noradrenaline, although adrenaline release was also increased significantly. This confirms the observation that the pattern of stimulation determines the pool of chromaffin cells from which catecholamines are derived and hence the adrenaline:noradrenaline ratio (Edwards, 1982). However, in that previous study it was adrenaline, and not noradrenaline, whose relative release was apparently potentiated by stimulation in bursts. As the present experimental conditions and the ages of the animals were practically identical with those which were obtained in that series of experiments the most likely explanation for the difference is to be found in the analytical techniques. The HPLC separation and determination of catecholamines used here is likely to be much more reliable than the fluorimetric determination employed in the earlier study.

Several workers have reported the presence of dopamine in both the cortex and medulla of the adrenal gland (Waldeck, Snider, Brown & Carlsson, 1975; Van Loon & Sale, 1980; Lackovic & Relja, 1984; Jones, Roebuck, Walker, Lagercrantz & Johnston, 1987) and it has been found to be released from sheep adrenals (Lishajko, 1968, 1969) although there appear to be no previous reports that it is released in response to splanchnic nerve stimulation. That is clearly established by the present results, as is the fact that the effect is potentiated by stimulating in bursts. The maximal rates of production are, however, about 100-fold below those for adrenaline and noradrenaline. In spite of this comparatively low rate of dopamine release, it would account for a significant proportion of that stored in the gland, taken over the whole 10 min period of stimulation (Waldeck *et al.* 1975; Van Loon & Sale, 1980; Lackovic & Relja, 1984; Jones *et al.* 1987). Some of the dopamine is present in the cortex and that could account for a proportion of the release, but larger amounts are found in the medulla and most of that appears to be localized within the nerve terminals rather than the chromaffin granules. This probably explains why section of the splanchnic nerve or treatment with 6-hydroxydopamine reduces the adrenal dopamine content (Poggioli, Solier & Beauvallet, 1975; Lackovic, Relja & Neff,

1981; Lackovic & Relja, 1984). Intraneuronal localization of the medullary, and possibly cortical, dopamine (Lackovic & Relja, 1984) would be consistent with the relatively low rates of release encountered during splanchnic nerve stimulation together with the fact that release was potentiated by stimulating in bursts. The high DOPAC:dopamine ratio in the adrenal venous effluent plasma is also noteworthy, together with the close correlation between DOPAC and dopamine release, because this has been taken as evidence that the amine is fulfilling a neurotransmitter role, rather than merely providing precursor, in other tissues (Bacopoulos, Hattox & Roth, 1979; Lackovic, Relja & Neff, 1982; Arkinstall & Jones, 1985).

Even though the amounts of VIP that were released from the gland were so tiny in response to both patterns of splanchnic nerve stimulation, the fact that it is released at all answers an important question. All the available evidence suggests that this peptide acts exclusively as a transmitter close to the site of release and since the VIP-ergic innervation that has been described in the adrenal cortex is sparse and the gland has a relatively high blood flow one would not expect to find more than a trace being washed out. Thus, identification of that trace confirms the fact that it is being released at a time when it could be acting on the adrenal cortical cells to potentiate their steroidogenic response to ACTH and so supports that suggestion (Edwards & Jones, 1987; Bloom *et al.* 1987*b*). VIP is known to exert a steroidogenic effect on adrenal cortical cells *in vitro* (Kowal, Horst, Pensky & Alfonzo, 1977; Morera, Cathiard, Laburthe & Saez, 1979; Leboulenger, Leroux, Delarue, Tonon, Charnay, Dubois, Coy & Vaudry, 1983), and the ovary (Frederichs, Lundquist, Mathur, Ashton & Landgrebe, 1983; Davoren & Hsueh, 1985; Ahmed, Dees & Ojeda, 1986), in which it has been attributed to increased synthesis of the ovarian cholesterol side-chain cleavage enzyme complex, which represents the rate-limiting reaction in progesterone synthesis, and is mediated, at least in part, via cyclic AMP (Trzeciak, Ahmed, Simpson & Ojeda, 1986). It is also noteworthy, in this connection, that whereas VIP-ergic fibres are confined to the zona glomerulosa in rat adrenal cortex, they have recently been identified in other zones of the cortex in sheep (Cheung & Holzwarth, 1986), making it the more likely that they are implicated in potentiating the steroidogenic effect of ACTH.

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