J. Physiol. (1984), **346**, *pp.* 519–531
With 10 *text-figures*
Printed in Great Britain

NEUROENDOCRINE RESPONSES TO STIMULATION OF THE SPLANCHNIC NERVES IN BURSTS IN THE CONSCIOUS ADRENALECTOMIZED CALF

BY S. R. BLOOM*, A. V. EDWARDS AND M. A. GHATEI*

*From the *Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, Ducane Road, London W12, and the Physiological Laboratory, University of Cambridge, Cambridge CB2 3EG*

(*Received 14 April 1983*)

SUMMARY

1. Effects of stimulation of the peripheral ends of the splanchnic nerves below behavioural threshold at either 4 or 2 Hz continuously for 10 min, and at 40 or 20 Hz for 1 s at 10 s intervals for 10 min, have been compared in conscious calves.

2. Cardiovascular responses were apparently unaffected by the pattern of the stimulus, whereas pancreatic neuroendocrine responses were significantly enhanced by stimulation in bursts, as was the rise in mean arterial plasma glucose concentration.

3. Release of bombesin-like immunoreactivity was substantially potentiated by intermittent stimulation at relatively high frequencies and the significance of this discovery is discussed in relation to the effects that this peptide is known to evoke in this species.

INTRODUCTION

Recent studies of the effect of stimulating autonomic nerves intermittently in bursts have shown that it potentiates the responses of certain effectors whereas others are relatively insensitive to this pattern of stimulation (for reviews see Edwards & Bloom, 1982; Edwards, Järhult, Andersson & Bloom, 1982). In the course of this work it has been found that the release of adrenaline, but not noradrenaline, from the adrenal medulla is significantly enhanced in conscious calves by stimulation of the peripheral end of the splanchnic nerve in bursts (Edwards, 1982).

The present study was undertaken to discover whether other neuroendocrine responses to splanchnic nerve stimulation could be modified by stimulation in bursts. The effects of stimulation at either 40 or 20 Hz for 1 s at 10 s intervals for 10 min were compared with those of continuous stimulation at 4 or 2 Hz for the same period, so that the total number of impulses delivered was identical in the two groups. The experiments were carried out in conscious adrenalectomized calves, thereby eliminating any non-specific effects due to anaesthetic agents and secondary responses due to release of catecholamines from the adrenal medullae.

Certain of these results have been published previously in a preliminary form (Bloom & Edwards, 1982).

METHODS

Animals

Pedigree Jersey calves were obtained from local farms shortly after birth and used at ages ranging between 19 and 47 days (28·1–41·5 kg body weight). Thereafter they were kept in individual pens in the laboratory animal house and maintained on a diet of either cow's milk or artificial milk (Easy-Mix Volac, Volac Ltd) at a rate of 2–4 l/day. Food was withheld for at least 14 h before surgery and each experiment.

Experimental procedures

Preparatory surgical and experimental procedures were identical with those described previously (Bloom & Edwards, 1980*a*), both splanchnic nerves being cut immediately below the diaphragm and both adrenal glands removed. Following adrenalectomy steroid replacement therapy was instituted by the administration of desoxycortisone acetate (0·2 mg/kg) and cortisol (10·0 mg/kg) by intramuscular injection.

The splanchnic nerves were stimulated using a standard 10–20 square-wave stimulus (pulse width 0·5 ms) at either 2 or 4 Hz continuously for 10 min, or at 20 or 40 Hz for 1 s at 10 s intervals for the same period.

Estimations

Samples of arterial blood and intestinal lymph were collected into heparinized tubes containing aprotinin (Trasylol, Bayer; 1000 K.I.U./ml blood) for peptide assays. These tubes were centrifuged at +4 °C immediately and the supernatant stored at −20 °C. Plasma glucose concentration was estimated with glucose oxidase by means of a Beckman 2 Glucose Analyzer and intestinal lymph flow was measured gravimetrically.

All peptide hormones were measured by radioimmunoassay (Bloom & Long, 1982). Pancreatic glucagon was assayed using an antiserum relatively specific for pancreatic glucagon which was C-terminal reacting (Assan & Slusher, 1972) and gave zero values in human plasma after total pancreatectomy, reacting less than 5% with 'glucagon-like immunoreactivity of ileal origin' (enteroglucagon). Insulin, pancreatic polypeptide (PP), neurotensin and vasoactive intestinal peptide (VIP) were also measured by radioimmunoassays that we have employed routinely in the past (Albano, Ekins, Maritz & Turner, 1972; Adrian, Bloom, Bryant, Polak, Heitz & Barnes, 1976; Mitchell & Bloom, 1978; Blackburn & Bloom, 1979).

Somatostatin (SRIF) was estimated using an antiserum raised in a rabbit to ovine somatostatin-14. The Tyr-11 analogue was iodinated with iodine-125 using lactoperoxidase. The assay detected differences in plasma somatostatin between individual samples of 4 pmol/l with 95% confidence. It fully cross-reacted with somatostatin-28 but reacted poorly or not at all with fragments or shortened analogues.

Gastric inhibitory peptide-like immunoreactivity (GIP) was measured using an antiserum raised to the pure natural porcine peptide in rabbits. Natural GIP was iodinated by lactoperoxidase. The assay also detected 'big' GIP but did not show any cross-reaction with glucagon, secretin or VIP. Differences of 3 pmol/l plasma could be detected between individual samples with 95% confidence (Sarson, Bryant & Bloom, 1980). Gastrin releasing peptide (GRP), or mammalian bombesin-like immunoreactivity (BLI), was measured with an antiserum raised to synthetic Lys^3-bombesin conjugated with glutaraldehyde to bovine serum albumin in rabbits. The radioactive assay label was prepared, using a Tyr^5-bombesin C-terminal nonapeptide analogue, by chloramine-T oxidation and iodine-125. This assay detects bombesin and pure porcine GRP with equal potency and is capable of distinguishing changes in plasma bombesin-like immunoreactivity of 5 pmol/l with 95% confidence (Ghatei, Jung, Stevenson, Hillyard, Adrian, Lee, Christofides, Sarson, Mashiter, MacIntyre & Bloom, 1982).

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

RESULTS

Responses to stimulation at 4 Hz continuously for 10 min and at 40 Hz for 1 s at 10 s intervals for the same period

Cardiovascular responses. No significant difference was observed between the cardiovascular responses of the two groups of animals to these two patterns of stimulation, as is illustrated in Fig. 1. In both groups the mean aortic blood pressure rose by about 40 mmHg, mean heart rate fell by about 35 beats/min and mean

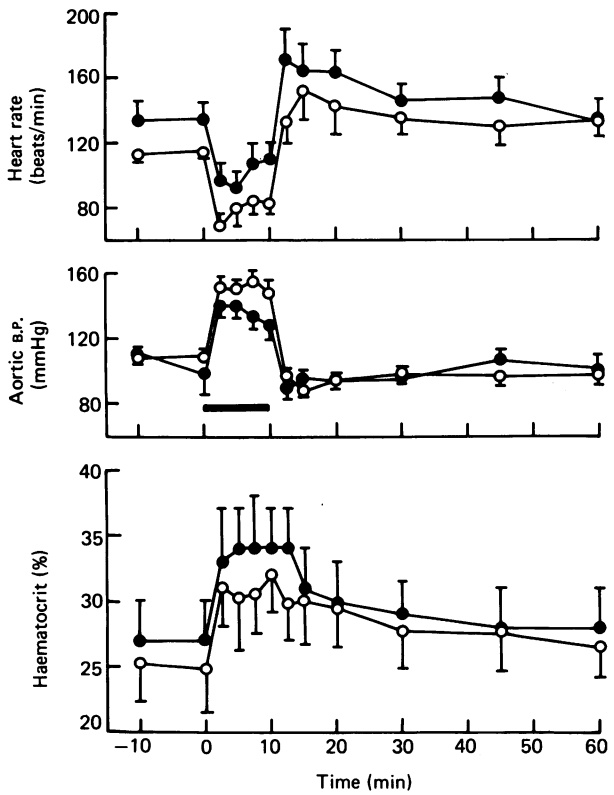

Fig. 1. Comparison of the changes in mean aortic blood pressure (B.P.), heart rate and haematocrit in conscious adrenalectomized 3–6-week-old calves in response to stimulation of the peripheral ends of the splanchnic nerves, at either 4 Hz continuously for 10 min (●; $n = 8$) or 40 Hz for 1 s at 10 s intervals for the same period (○; $n = 6$). Horizontal bar, duration of stimulus. Vertical bars, S.E. of each mean value.

haematocrit rose by about 7 %. Nor was there any statistically significant difference between the initial values recorded in the two groups in respect of any of these parameters.

Pancreatic endocrine responses. Unlike the cadiovascular responses, the release of glucagon and insulin were both found to be significantly affected by the pattern of splanchnic nerve stimulation. Stimulation in bursts elicited a greater rise in mean plasma pancreatic glucagon concentration (to a peak incremental value of

817 ± 49 pmol/l) than continuous stimulation at the corresponding lower frequency (548 ± 61 pmol/l; $P < 0.01$) (Fig. 2). Stimulation in bursts also caused a significantly greater suppression of insulin release, as assessed by the changes in mean plasma insulin concentration ($P < 0.05$). These differences were associated with a highly significant difference between the hyperglycaemic responses ($P < 0.001$); the rise in

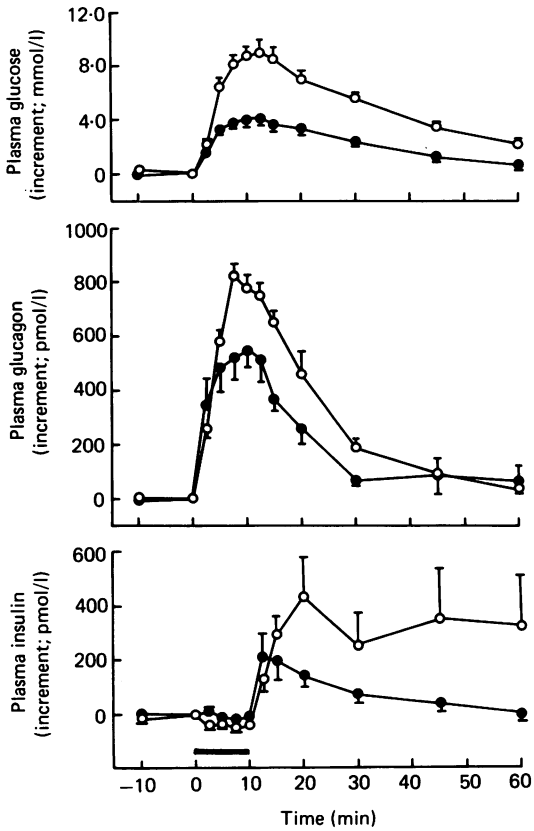

Fig. 2. Comparison of the changes in mean arterial plasma glucose, pancreatic glucagon and insulin concentration in 3–6-week-old conscious adrenalectomized calves in response to stimulation of the peripheral ends of the splanchnic nerves, at either 4 Hz continuously for 10 min (●; $n = 8$) or 40 Hz for 1 s at 10 s intervals for the same period (○; $n = 6$). Horizontal bar, duration of stimulus. Vertical bars, S.E. of each mean value where these exceed the size of the symbol. Absolute values at time 0 in the group stimulated at 4 Hz continuously: glucose, 4·3 ± 0·4 mmol/l; glucagon, 35 ± 8 pmol/l; insulin, 43 ± 15 pmol/l. In the group stimulated at 40 Hz in bursts: glucose, 4·5 ± 0·3 mmol/l; glucagon, 26 ± 8 pmol/l; insulin, 66 ± 18 pmol/l.

mean arterial plasma glucose concentration in response to stimulation in bursts was found to be more than double that which occurred in response to continuous stimulation (Fig. 2). In contrast, the rise in mean arterial plasma pancreatic polypeptide (PP) concentration was not significantly affected by the pattern of stimulation (Fig. 3).

Release of gastrointestinal peptides. The change in the concentration of VIP in intestinal lymph is a more reliable index of release of the peptide from the gastrointestinal tract in response to autonomic stimulation than is a change in the concentration in the arterial plasma (Bloom & Edwards, 1980*b*). Accordingly, the changes in mean lymphatic VIP concentration were monitored in preference to those in the arterial plasma, together with the flow of intestinal lymph. Maintenance of

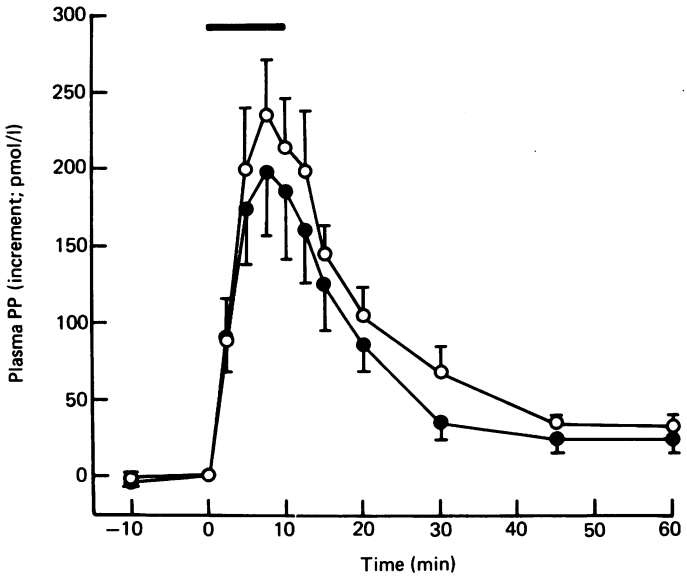

Fig. 3. Comparison of the changes in mean arterial plasma PP concentration in 3–6-week-old conscious adrenalectomized calves in response to stimulation of the peripheral ends of the splanchnic nerves, at either 4 Hz continuously for 10 min (●; $n = 8$) or 40 Hz for 1 s at 10 s intervals for the same period (○; $n = 6$). Horizontal bar, duration of stimulus. Vertical bars, S.E. of each mean value. Absolute values at time 0 in the group stimulated at 4 Hz continuously: 58 ± 6 pmol/l. In the group stimulated at 40 Hz in bursts: 44 ± 5 pmol/l.

patent lymphatic catheters in conscious animals is associated with much greater technical difficulties than the other preparatory surgical procedures necessitated by this study, and the number of successful experiments in which lymph could be collected was therefore less. The changes in mean lymphatic VIP concentration were also relatively small, and the differences were not statistically significant and are not permissive of any definite conclusions. However, it is noteworthy that the mean lymphatic VIP concentration was raised by about 4 pmol/l throughout the period of stimulation in bursts and reduced by about the same amount during continuous stimulation (Fig. 4*B*). Stimulation in bursts also seemed to be more effective in raising the flow of lymph (Fig. 4*A*), but larger groups of animals tested at higher stimulus frequencies would be needed to establish both these observations with certainty. No evidence was obtained in any of these experiments which conflicted with the conclusion that changes in VIP output from the gastrointestinal tract in response to direct stimulation of the autonomic innervation merely reflect 'wash-out' of a peptidergic transmitter.

Release of bombesin-like immunoreactivity (BLI) from the gastrointestinal tract was, however, substantially potentiated by stimulation in bursts. The mean peak incremental value at 10 min was 170±22 pmol/l in response to stimulation in bursts at 40 Hz for 1 s at 10 s intervals,whereas that during continuous stimulation at 4 Hz was 42±10 pmol/l ($P < 0.001$; Fig. 5*A*). A further intriguing feature of the effects

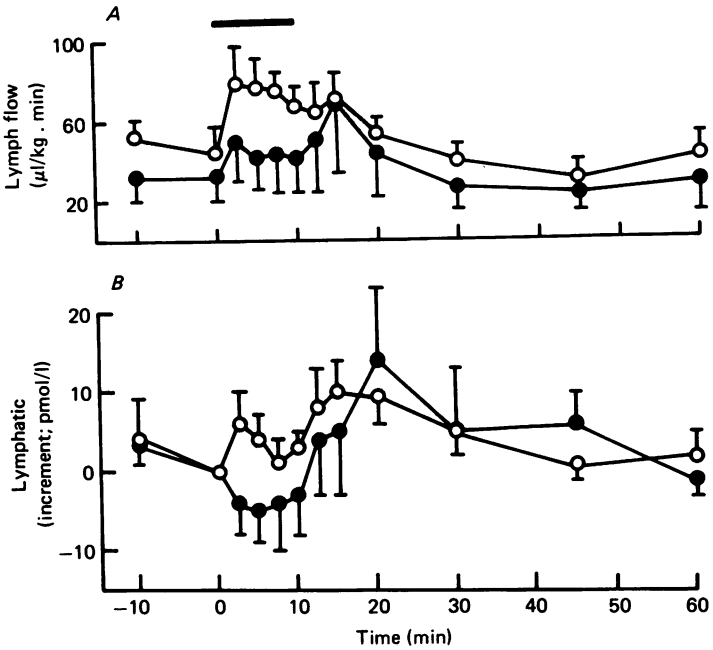

Fig. 4. Comparison of the changes in the mean flow of intestinal lymph (*A*) and concentration of VIP therein (*B*) of 3–6-week-old conscious adrenalectomized calves in response to stimulation of the peripheral ends of the splanchnic nerves at either 4 Hz continuously for 10 min (●; $n = 4$) or 40 Hz for 1 s at 10 s intervals for the same period (○; $n = 7$). Horizontal bar, duration of stimulus. Vertical bars, S.E. of each mean value. Absolute VIP concentration at time 0 in the group stimulated at 4 Hz continuously: 22±8 pmol/l. In the group stimulated at 40 Hz in bursts: 8±2 pmol/l.

on the release of BLI of these two patterns of splanchnic nerve stimulation is the fact that the mean peak incremental value in the intestinal lymph (264±30 pmol/l, which occurred at 12·5 min due to the relatively slow rate of flow of intestinal lymph and the irreducible dead-space of the lymphatic system) in response to stimulation in bursts significantly exceeded that in the arterial plasma ($P < 0.05$, Fig. 5*B*). In contrast the changes in mean plasma and lymphatic BLI were closely similar when the nerves were stimulated at the corresponding lower frequency (Fig. 5*C*).

Both patterns of stimulation elicited closely similar changes in the mean arterial plasma concentration of gastric inhibitory peptide-like immunoreactivity (GIP) and neurotensin-like immunoreactivity (NTI). In the case of the former, a small rise was recorded and in the case of the latter a small fall; we have no reason to suppose that either of these changes is of physiological significance (Figs. 6 and 7).

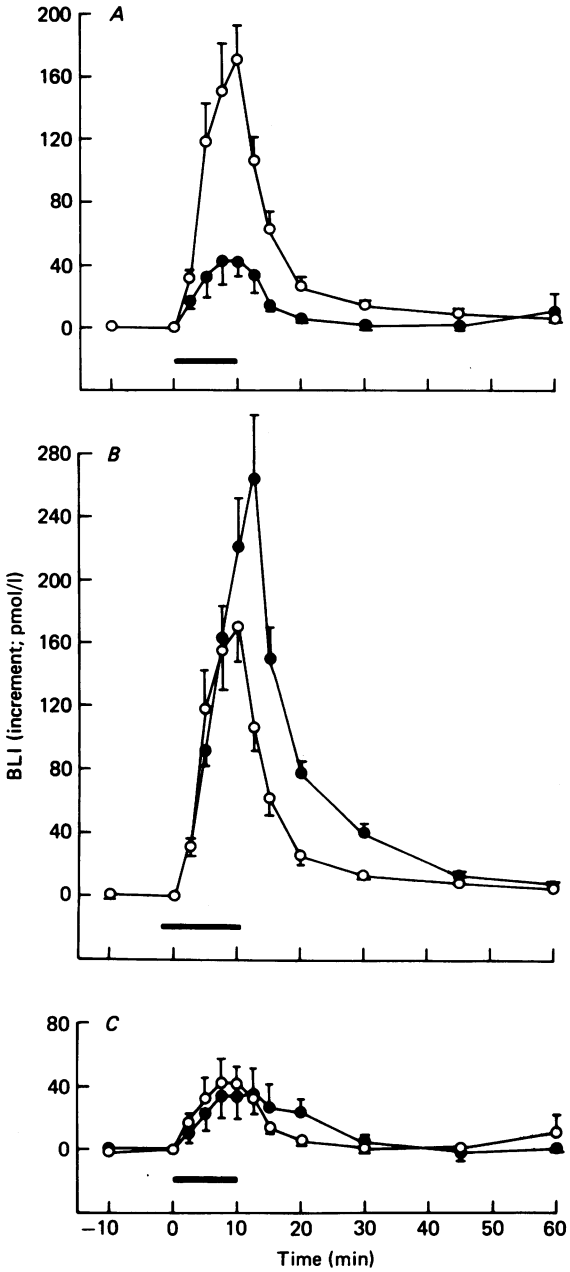

Fig. 5. Changes in mean arterial plasma and intestinal BLI in conscious 3–6-week-old adrenalectomized calves in response to stimulation of the peripheral ends of the splanchnic nerves continuously at 4 Hz for 10 min, or at 40 Hz for 1 s at 10 s intervals for the same period. Horizontal bar, duration of stimulus. Vertical bars, S.E. of each mean value. *A*, the differences in plasma BLI in response to continuous stimulation (●; $n = 8$; absolute mean value at time 0, 21 ± 5 pmol/l) and stimulation in bursts (○; $n = 6$; absolute mean value at time 0, 7 ± 2 pmol/l). *B*, the changes in mean plasma (○; $n = 6$) and lymphatic (●; $n = 5$) BLI in response to stimulation in bursts.The initial mean value for lymphatic BLI at time 0 was 5 ± 1 pmol/l. *C*, the changes in mean plasma (○; $n = 8$) and lymphatic BLI (●; $n = 5$) in response to continuous stimulation. The initial mean value for lymphatic BLI was 14 ± 6 pmol/l.

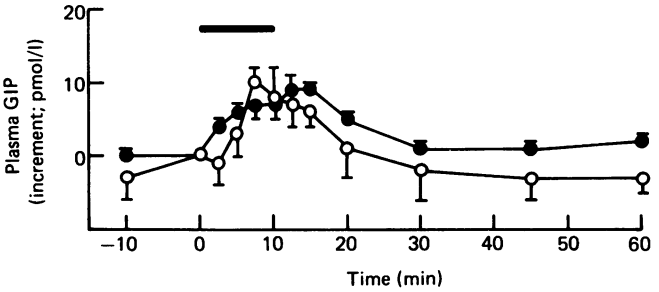

Fig. 6. Comparison of the changes in mean GIP-like immunoreactivity in the arterial plasma of 3–6-week-old conscious adrenalectomized calves in response to stimulation of the peripheral ends of the splanchnic nerves, at either 4 Hz continuously for 30 min (●; $n = 8$) or 40 Hz for 1 s at 10 s intervals for the same period (○; $n = 6$). Horizontal bar, duration of stimulus. Vertical bars, S.E. of each mean value. Absolute values at time 0 in the group stimulated at 4 Hz continuously: 13±2 pmol/l. In the group stimulated at 40 Hz in bursts: 18±6 pmol/l.

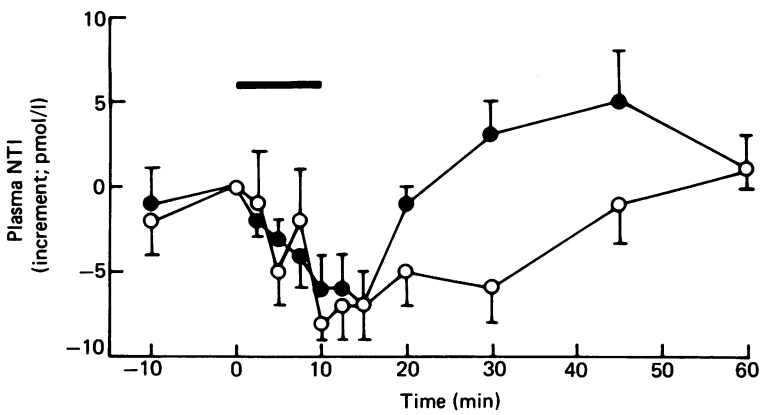

Fig. 7. Comparison of the changes in mean arterial plasma NTI-like immunoreactivity in conscious 3–6-week-old adrenalectomized calves in response to stimulation of the peripheral ends of the splanchnic nerves, either at 4 Hz continuously for 10 min (●; $n = 8$) or at 40 Hz for 1 s at 10 s intervals for the same period (○; $n = 6$). Horizontal bar, duration of stimulus. Vertical bars, S.E. of each mean value. Absolute values at time 0 in the group stimulated at 4 Hz continuously: 13±3 pmol/l. In the group stimulated at 40 Hz in bursts: 11±3 pmol/l.

Neither pattern of stimulation produced a significant change in mean arterial plasma somatostatin-like immunoreactivity (SRIF), but plasma SRIF rose to significantly higher levels after continuous stimulation than it did following stimulation in bursts ($P < 0{\cdot}02$; Fig. 8).

Responses to stimulation at 2 Hz continuously for 10 min and at 20 Hz for 1 s at 10 s intervals for the same period

Those responses that were significantly enhanced by stimulation in bursts at 40 Hz, by comparison with the corresponding continuous stimulus frequency, were also monitored during stimulation at a lower frequency range (20 Hz in bursts and 2 Hz

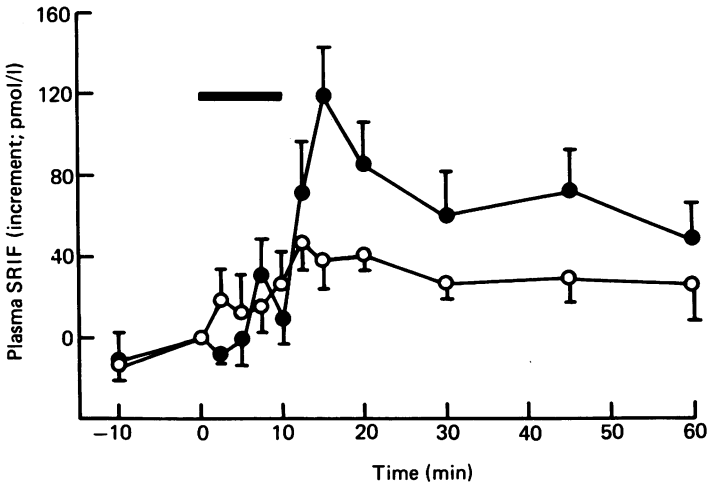

Fig. 8. Comparison of the changes in mean arterial plasma SRIF in conscious 3–6-week-old adrenalectomized calves in response to stimulation of the peripheral ends of the splanchnic nerves, either at 4 Hz continuously for 10 min (●; $n = 8$) or at 40 Hz for 1 s at 10 s intervals for the same period (○; $n = 6$). Horizontal bar, duration of stimulus. Vertical bars, S.E. of each mean value. Absolute values at time 0 in the group stimulated at 4 Hz continuously: 65 ± 22 pmol/l. In the group stimulated at 40 Hz in bursts: 56 ± 8 pmol/l.

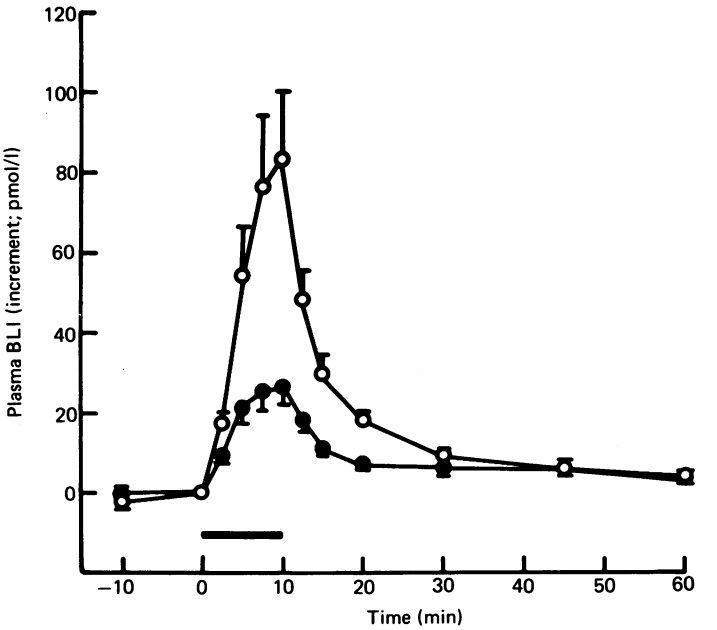

Fig. 9. Comparison of the changes in mean arterial plasma BLI in conscious 3–6-week-old adrenalectomized calves in response to stimulation of the peripheral ends of the splanchnic nerves, either at 2 Hz continuously for 10 min (●; $n = 9$) or at 20 Hz for 1 s at 10 s intervals for the same period (○; $n = 9$). Horizontal bar, duration of stimulus. Vertical bars, S.E. of each mean value. Absolute values at time 0 in the group stimulated at 2 Hz continuously: 33 ± 3 pmol/l. In the group stimulated at 20 Hz in bursts: 35 ± 4 pmol/l.

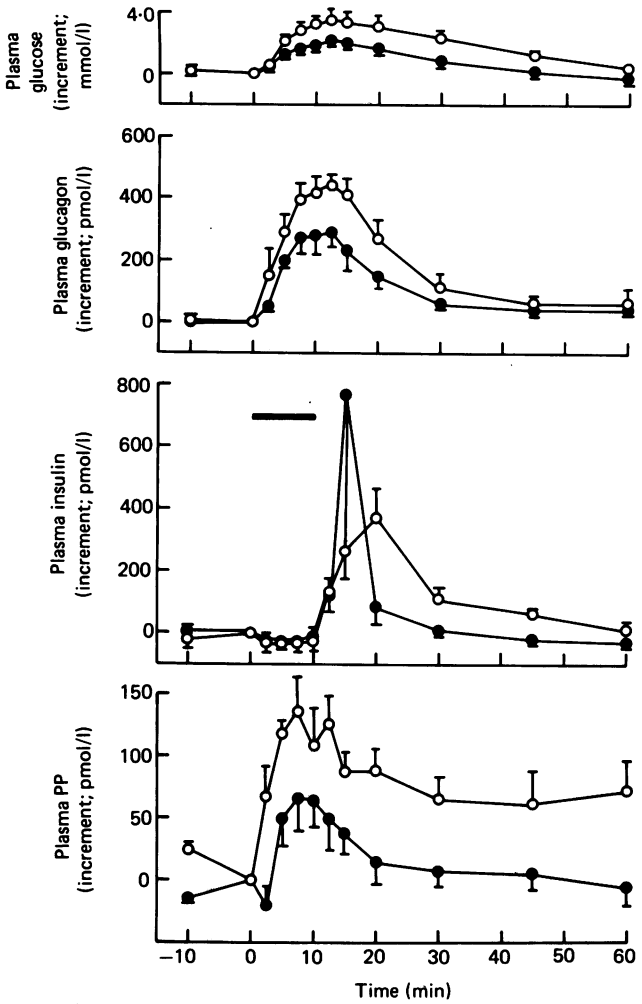

Fig. 10. Comparison of the changes in mean arterial plasma glucose, pancreatic glucagon, insulin and PP concentration in 3–6-week-old conscious adrenalectomized calves in response to stimulation of the peripheral ends of the splanchnic nerves, either at 2 Hz continuously for 10 min (●; $n = 9$) or at 20 Hz for 1 s at 10 s intervals for the same period (○; $n = 9$). Horizontal bar, duration of stimulus. Vertical bars, S.E. of each mean value. Absolute values at time 0 in the group stimulated at 2 Hz continuously: glucose, $3{\cdot}7 \pm 0{\cdot}4$ mmol/l; glucagon, 23 ± 6 pmol/l; insulin, 55 ± 16 pmol/l; PP, 90 ± 20 pmol/l. In the group stimulated at 20 Hz in bursts: glucose, $3{\cdot}5 \pm 0{\cdot}3$ mmol/l; glucagon, 45 ± 15 pmol/l; insulin, 56 ± 31 pmol/l; PP, 108 ± 14 pmol/l.

continuously) in order to determine whether the enhancement existed over a range of frequencies. The results of these experiments showed that the rise in mean arterial plasma BLI was also significantly potentiated by stimulation in bursts under these conditions. The mean peak incremental value at 10 min in response to stimulation at 20 Hz for 1 s at 10 s intervals was 83 ± 17 pmol/l, while the corresponding peak

value at the same experimental time in response to stimulation at 2 Hz continuously was 26 ± 4 pmol/l ($P < 0{\cdot}01$; Fig. 9).

The patterns of the pancreatic endocrine responses were also similar to those observed at higher frequencies (Figs. 2 and 10), although the peak incremental values only achieved statistical significance in the case of pancreatic glucagon ($P < 0{\cdot}01$). The rise in mean arterial plasma PP concentration was found to be significantly greater in response to stimulation in bursts when the paired t test was applied individually to the areas under the curve ($P < 0{\cdot}05$).

DISCUSSION

The results of these experiments, which were devised to investigate the possibility that the pattern of stimulation might be a determinative factor in relation to certain responses to stimulation of the splanchnic sympathetic innervation, indicate that this is true in respect of some, but certainly not all such responses. It is worth noting that the sympathetic splanchnic innervation differs from the vagal splanchnic innervation in this respect, as no significant differences emerged from a precisely similar study of the effects of different patterns of vagal stimulation in animals of the same age and species (Adrian, Bloom & Edwards, 1983).

The fact that certain post-ganglionic sympathetic neurones normally fire in bursts intermittently at relatively high frequencies has been established by the classical studies of Wallin and his colleagues, which show that this is characteristic of fibres supplying both skin and skeletal muscle in unanaesthetized human subjects (Wallin, 1981). The further fact that enhancement of autonomic responses by stimulation in bursts at high frequencies is not necessarily a consequence of improved ganglionic transmission is supported by the finding that certain responses to stimulation of the autonomic innervation are potentiated when the post-ganglionic neurones are stimulated directly. Examples of this phenomenon are provided by recent studies on the submaxillary gland of the cat (Andersson, Bloom, Edwards & Järhult, 1982) and the parotid gland of the weaned lamb (Andersson, Bloom & Edwards, 1982).

The results of the present study show that, whereas cardiovascular responses seem to be unaffected by the pattern of stimulation that is applied to the splanchnic sympathetic innervation, pancreatic neuroendocrine responses are highly susceptible to the pattern of the stimulus. This is demonstrable in relation to release of pancreatic glucagon and PP, and suppression of insulin release.

The most dramatic difference in the various responses to these different patterns of stimulation that we were able to monitor was release of BLI. It seems to be generally accepted that this peptide is only present in nerve terminals in the gastrointestinal tract (Dockray, Vaillant & Walsh, 1979) and it can therefore be considered to be a putative transmitter or paracrine agent. In the young calf, this contention is supported by the finding that BLI is present in much larger amounts in the smooth muscle of the gastrointestinal tract than the mucosa (Bloom, Edwards & Ghatei, 1983), and by the fact that its release in response to splanchnic nerve stimulation persists in the presence of propranolol, phentolamine and atropine but is abolished by hexamethonium (Bloom & Edwards, 1984). The recent discovery that

intravenous infusions of the synthetic peptide (at doses which reproduce the rise in plasma BLI which we have recorded in response to splanchnic nerve stimulation in bursts at 40 Hz; Bloom *et al.* 1983) produce substantial and significant biological responses suggests that this peptide may well exert both local and generalized effects, such as we have not envisaged previously.

This work was supported by grants from the British Diabetic Association and the Science and Engineering Research Council. It is a particular pleasure to thank Messrs P. M. M. Bircham and G. P. McGregor for their skilled technical assistance.

REFERENCES

ADRIAN, T. E., BLOOM, S. R., BRYANT, M. G., POLAK, J. M., HEITZ, P. H. & BARNES, A. J. (1976). Distribution and release of human pancreatic polypeptide. *Gut* **17**, 940–944.

ADRIAN, T. E., BLOOM, S. R. & EDWARDS, A. V. (1983). Neuroendocrine responses to stimulation of the vagus nerves in bursts in conscious calves. *J. Physiol.* **344**, 25–35.

ALBANO, J. D., EKINS, R. P., MARITZ, G. & TURNER, R. C. (1972). Sensitive precise radioimmunoassay of serum insulin relying on charcoal separation of bound and free hormone moieties. *Acta endocr., Copenh.* **70**, 487–509.

ANDERSSON, P.-O., BLOOM, S. R. & EDWARDS, A. V. (1982). Parotid responses to stimulation of the parasympathetic innervation in bursts in weaned lambs. *J. Physiol.* **330**, 163–174.

ANDERSSON, P.-O., BLOOM, S. R., EDWARDS, A. V. & JÄRHULT, J. (1982). Effects of stimulation of the chorda tympani in bursts on submaxillary responses in the cat. *J. Physiol.* **322**, 469–483.

ASSAN, R. & SLUSHER, N. (1972). Structure/function and structure/immunoreactivity relationships of the glucagon molecule and related synthetic peptides. *Diabetes* **21**, 843–855.

BLACKBURN, A. M. & BLOOM, S. R. (1979). A radioimmunoassay for neurotensin in human plasma. *J. Endocr.* **83**, 409–419.

BLOOM, S. R. & EDWARDS, A. V. (1980*a*). Pancreatic endocrine responses to stimulation of the peripheral ends of the splanchnic nerves in the conscious adrenalectomized calf. *J. Physiol.* **308**, 39–48.

BLOOM, S. R. & EDWARDS, A. V. (1980*b*). Effects of autonomic stimulation on the release of vasoactive intestinal peptide from the gastrointestinal tract in response to autonomic stimulation in the calf. *J. Physiol.* **299**, 437–452.

BLOOM, S. R. & EDWARDS, A. V. (1982). Neuroendocrine responses to stimulation of the peripheral ends of the splanchnic nerves in bursts in the conscious adrenalectomized calf. *J. Physiol.* **332**, 84–85*P*.

BLOOM, S. R. & EDWARDS, A. V. (1984). Characteristics of the neuroendocrine responses to stimulation of the splanchnic nerves in bursts in the conscious calf. *J. Physiol.* **346**, 533–545.

BLOOM, S. R., EDWARDS, A. V. & GHATEI, M. A. (1983). Endocrine responses to exogenous bombesin and gastrin releasing peptide in conscious calves. *J. Physiol.* **344**, 37–48.

BLOOM, S. R. & LONG, R. G. (1982). *Radioimmunoassay of Gut Regulatory Peptides*. Philadelphia: W. B. Saunders.

DOCKRAY, G. J., VAILLANT, C. & WALSH, J. H. (1979). The neuronal origin of bombesin-like immunoreactivity in the rat gastrointestinal tract. *Neuroscience* **4**, 1561–1568.

EDWARDS, A. V. (1982). Adrenal catecholamine output in response to stimulation of the splanchnic nerve in bursts. *J. Physiol.* **327**, 409–419.

EDWARDS, A. V. & BLOOM, S. R. (1982). Recent physiological studies of the alimentary autonomic innervation. *Scand. J. Gastroenterol.* **17**, suppl. 71, 77–89.

EDWARDS, A. V., JÄRHULT, J., ANDERSSON, P.-O. & BLOOM, S. R. (1982). The importance of the pattern of the stimulus in relation to the response of autonomic effectors. In *Systemic Role of Regulatory Peptides, Symposia Medica Hoechst* 18, ed. BLOOM, S. R., POLAK, J. M. & LINDENLAUB, E., pp. 145–168. Stuttgart & New York: Schattauer Verlag.

GHATEI, M. A., JUNG, R. T., STEVENSON, J. C., HILLYARD, C. J., ADRIAN, T. E., LEE, Y. C., CHRISTOFIDES, N. C., SARSON, D. L., MASHITER, K., MACINTYRE, I. & BLOOM, S. R. (1982). Bombesin: action on gut hormones and calcium in man. *J. clin. endocr. Metab.* **54**, 980–985.

MITCHELL, S. J. & BLOOM, S. R. (1978). Measurement of fasting and postprandial VIP in man. *Gut* **19**, 1043–1048.

SARSON, D. L., BRYANT, M. G. & BLOOM, S. R. (1980). A radioimmunoassay for gastric inhibitory polypeptide in human plasma. *J. Endocr.* **85**, 487–495.

SNEDECOR, G. W. & COCHRAN, W. G. (1967). *Statistical Methods*, 6th edn. Ames: Iowa State College Press.

WALLIN, B. G. (1981). New aspects of sympathetic function in man. In *Int. Med. Rev. Neurology*, vol. 1, *Clinical Neurophysiology*, ed. STÅLBERG, E. & YOUNG, R. R., pp. 145–167. London: Butterworth.