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

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SUMMARY

1. Neuroendocrine responses to splanchnic nerve stimulation in bursts (at 40 Hz for 1 s at 10 s intervals for 10 min) have been investigated in conscious adrenalectomized calves, 3–6 weeks after birth, in the presence of various pharmacological blocking agents.

2. Preganglionic blockade with hexamethonium abolished all the neuroendocrine responses that were monitored.

3. Pre-treatment with phentolamine significantly reduced, but failed to eliminate, the release of both pancreatic glucagon and pancreatic polypeptide. In the presence of phentolamine splanchnic nerve stimulation produced a massive rise in arterial plasma insulin concentration. None of these pancreatic neuroendocrine responses was significantly affected by additional pre-treatment with propanolol.

4. The rise in mean plasma insulin concentration which occurred in calves pre-treated with both phentolamine and propranolol was significantly reduced by atropine.

5. Release of bombesin-like immunoreactivity (BLI) was unaffected by total post-ganglionic adrenergic and cholinergic blockade.

6. The results indicate that pancreatic endocrine responses to splanchnic nerve stimulation may be attributable, at least in part, to release of BLI in this species.

INTRODUCTION

It is now well established that stimulation of the sympathetic innervation to the pancreas stimulates the release of glucagon (Esterhuizen & Howell, 1970; Bloom, Edwards & Vaughan, 1973; Marliss, Girardier, Seydoux, Wollheim, Kanazawa, Orci, Renold & Porte, 1973; Bloom & Edwards, 1975; Kaneto, Kajinuma & Kosaka, 1975) and inhibits release of insulin in spite of the concomitant rise in blood glucose concentration (Bloom *et al.* 1973; Bloom & Edwards, 1975; Girardier, Seydoux & Campfield, 1976; Järhult, Andersson, Holst, Moghimzadeh & Nobin, 1980). These findings have recently been confirmed in the conscious adrenalectomized calf and extended to show that stimulation of the peripheral ends of the splanchnic nerves

in this species also produces a substantial rise in the concentration of pancreatic polypeptide (PP) and bombesin-like immunoreactivity (BLI) in the arterial plasma (Bloom & Edwards, 1980, 1982). We have also reported the fact that certain of these responses are significantly potentiated by stimulating the nerves intermittently at relatively high frequencies (Bloom & Edwards, 1982).

With the exception of the cat, in which the inhibition of insulin release is blocked by propranolol (Andersson, Holst & Järhult, 1982), and is therefore presumably mediated via β-adrenoceptors, this response is invariably blocked by phentolamine, in the presence of which release of the hormone is strongly stimulated during splanchnic nerve stimulation (Kaneto *et al.* 1975; Miller, 1975; Bloom & Edwards, 1978). The release of pancreatic glucagon persists in the presence of phentolamine, propranolol and atropine in both the dog and the calf (Kaneto *et al.* 1975; Bloom & Edwards, 1978) but has been found to be substantially reduced by combined pre-treatment with α- and β-adrenergic blocking agents (Holst, Grønholt, Schaffalitzky de Muckadell & Fahrenkrug, 1981). Virtually nothing is known of the pharmacological characteristics of the release of either PP or BLI in response to splanchnic nerve stimulation. The experiments described in the present paper were undertaken in order to elucidate these processes in the calf; the splanchnic nerves were stimulated in bursts (40 Hz for 1 s at 10 s intervals) as this appears to be the optimum pattern of stimulation.

METHODS

Animals

Pedigree Jersey calves were obtained from local farms shortly after birth and used at ages ranging between 20 and 44 days (21·0–43·5 kg body weight). The animals were kept in individual pens 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 invariably withheld for at least 6 h before surgery and for 16 h before each experiment.

Experimental procedures

Anaesthesia was induced with chloroform (Chloroform SLR, Fisons) and maintained with halothane (halothane, May and Baker; *ca.* 2% in oxygen). Preparatory surgery involved bilateral adrenalectomy, or in some cases bilateral ligation of the adrenal veins, and the insertion of narrow-bore polyethylene catheters into the saphenous arteries and veins, so that the tips lay in the abdominal aorta and posterior vena cava respectively. These catheters were used subsequently to monitor aortic blood pressure and heart rate, to collect arterial blood samples for estimation of haematocrit, arterial plasma glucose and peptide concentrations, and for intravenous injections. The splanchnic nerves were cut bilaterally immediately below the diaphragm and the peripheral ends enclosed in fluid electrodes constructed of silver wire and silicone rubber designed to eliminate spread of stimulus. A standard 10–20 V square-wave stimulus (pulse width 0·5 ms) was invariably employed at a frequency of 40 Hz for 1 s at 10 s intervals for 10 min. Following surgery, adrenal steroid replacement was instituted by the administration of desoxycortisone acetate (0·2 mg/kg) and cortisol (10·0 mg/kg) by intramuscular injection.

When required, atropine (atropine sulphate, BDH) and hexamethonium (hexamethonium bromide, Koch-Light) were dissolved in a small volume of physiological saline and injected intravenously at doses of 0·2 and 10·0 mg/kg respectively, 10 min before the splanchnic nerves were stimulated. Propranolol (Inderal, ICI) was administered by a single intravenous injection at a dose of 0·25 mg/kg 10 min before stimulation. Animals treated with phentolamine (Rogitine, CIBA) were given a priming injection (0·1 mg/kg intravenously) 10 min before stimulation, followed immediately by continuous intravenous infusion at a dose of 0·02 mg/kg . min until 5 min after splanchnic nerve stimulation was discontinued.

Estimations

Samples of arterial blood and intestinal lymph were collected at intervals for peptide and glucose measurements. Aliquots destined for peptide assays were collected into heparinized tubes containing aprotinin (Trasylol, Bayer; 1000 K.I.U./ml blood) and centrifuged without delay at +4 °C; the plasma was subsequently stored at −20 °C. Glucose was measured enzymically by means of a Mark 2 Beckman Glucose Analyzer. Pancreatic glucagon was measured by a radioimmunoassay 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 and PP were also measured by radioimmunoassay (Albano, Ekins, Maritz & Turner, 1972; Adrian, Bloom, Bryant, Polak, Heitz & Barnes, 1976).

Gastrin-releasing peptide (GRP) or mammalian BLI were measured by 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 of plasma BLI 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

Comparison of responses to intermittent splanchnic nerve stimulation in calves pre-treated with either propranolol or phentolamine

Cardiovascular responses. Propranolol invariably produced a rapid fall in heart rate and the mean value at time 0 (87 ± 6 beats/min) was significantly less than that in the untreated control group (114 ± 5 beats/min, $P < 0.01$). This provided evidence of the efficacy of the dose employed and was in accord with the belief that the effects of the sympathetic innervation to the myocardium are mediated, at least in part, via β-adrenoceptors (Table 1). In contrast, there was no significant difference between the hypertensive effects of splanchnic nerve stimulation in these two groups of animals. Conversely, pre-treatment with phentolamine invariably produced a fall in aortic blood pressure and the mean value at time 0 (88 ± 5 mmHg) was significantly lower than the corresponding value in the control group (108 ± 5 mmHg, $P < 0.02$), which is in accordance with the proposition that sympathetic vasoconstrictor effects are mediated largely via α-adrenoceptors and shows that the dose of phentolamine employed induced effective blockade. The rise in mean aortic blood pressure was also substantially less in the presence of phentolamine than in the control group (Table 1). However, it is noteworthy that neither the basal haematocrit nor the rise that occurred in response to splanchnic nerve stimulation was significantly affected by either blocking agent, or even a combination of the two (Table 1).

Neuroendocrine responses. Phentolamine significantly reduced ($P < 0.01$), but failed to abolish, the rise in mean arterial plasma pancreatic glucagon concentration that occurs in response to splanchnic nerve stimulation at 40 Hz for 1 s at 10 s intervals, and this was associated with a reduction in the hyperglycaemic response (Fig. 1). Under the same conditions there was a massive rise in mean arterial plasma insulin concentration, which may have contributed to the reduced rise in mean plasma glucose concentration (Fig. 1).

The rise in mean arterial plasma PP concentration was also substantially reduced

TABLE 1. Changes in mean aortic blood pressure (B.P.), heart rate (H.R.) and haematocrit (Hct) in conscious 3 to 5-week-old calves in response to stimulation of the peripheral ends of the splanchnic nerves at 40 Hz for 1 s at 10 s intervals for 10 min in the presence and absence of adrenergic blocking agents

	Controls ($n = 6$)			+ Propranolol ($n = 8$)		
t (min)	B.P. (mmHg)	H.R. (beats/min)	Hct (%)	B.P. (mmHg)	H.R. (beats/min)	Hct (%)
−10	108±4	112±4	25·2±3·4	105±4	106±9	27·0±2·1
0	108±5	114±5	24·8±3·4	103±4	87±6	27·3±1·9
2½	151±6	68±8	31·0±3·0	144±6	54±5	32·1±2·3
5	150±5	79±10	30·2±3·9	147±5	50±6	36·0±1·9
7½	155±6	84±8	30·4±3·0	145±5	53±4	37·6±2·1
10	147±8	82±6	32·0±2·9	144±6	62±5	37·6±2·2
12½	97±4	133±14	29·8±2·8	98±4	94±7	37·0±2·2
15	87±2	152±16	30·0±3·3	84±4	101±6	33·6±3·1
20	94±3	142±17	29·4±2·9	85±4	95±4	30·8±2·2
30	98±4	135±10	27·8±2·9	91±4	88±4	29·3±1·5
45	97±5	130±12	27·6±2·8	90±5	85±4	29·1±1·8
60	98±6	134±12	26·6±2·5	86±5	85±5	28·4±1·8
	+ Phentolamine ($n = 8$)			+ Propranolol + phentolamine ($n = 7$)		
t (min)	B.P. (mmHg)	H.R. (beats/min)	Hct (%)	B.P. (mmHg)	H.R. (beats/min)	Hct (%)
−10	104±5	99±10	26·6±2·3	104±2	93±6	28·7±2·2
0	88±5	143±22	28·9±1·7	94±3	81±5	29·3±2·5
2½	110±2	92±10	37·1±1·8	121±5	62±6	35·6±2·5
5	101±3	128±13	38·9±1·4	113±3	68±6	35·4±2·8
7½	94±3	152±12	39·5±1·7	107±3	75±6	34·7±2·6
10	85±3	166±12	40·6±1·5	102±2	78±5	36·9±2·1
12½	81±5	131±13	38·4±2·1	77±3	85±4	32·8±2·2
15	80±3	123±10	38·5±1·4	82±4	83±3	32·5±2·7
20	82±4	120±6	37·0±1·9	84±3	84±5	32·0±3·3
30	82±4	149±18	35·6±1·8	84±3	85±5	33·1±3·1
45	84±5	141±15	33·9±1·9	80±4	85±7	32·1±3·3
60	96±9	143±17	32·6±1·4	85±4	86±6	32·6±2·7

in the presence of phentolamine (but not propranolol), although a significant rise in the mean concentration of PP in the arterial plasma was nevertheless recorded in the presence of phentolamine (Fig. 2).

The rise in mean arterial BLI was not significantly affected by pre-treatment with either phentolamine or propranolol under these conditions (Fig. 3).

Comparison of the responses of normal calves with those of calves pre-treated with a variety of pharmacological blocking agents

Cardiovascular responses. Combined treatment with propranolol and phentolamine produced a fall in mean heart rate and aortic blood pressure, and both were significantly lower at time 0 than the corresponding values in the control group ($P < 0·05$ and $P < 0·01$ respectively; Table 1). The changes in these parameters in response to splanchnic nerve stimulation were also attenuated by the combined action of these agents. Atropinized calves tested with the same agents responded to splanchnic nerve stimulation with a rise in mean aortic blood pressure that was closely

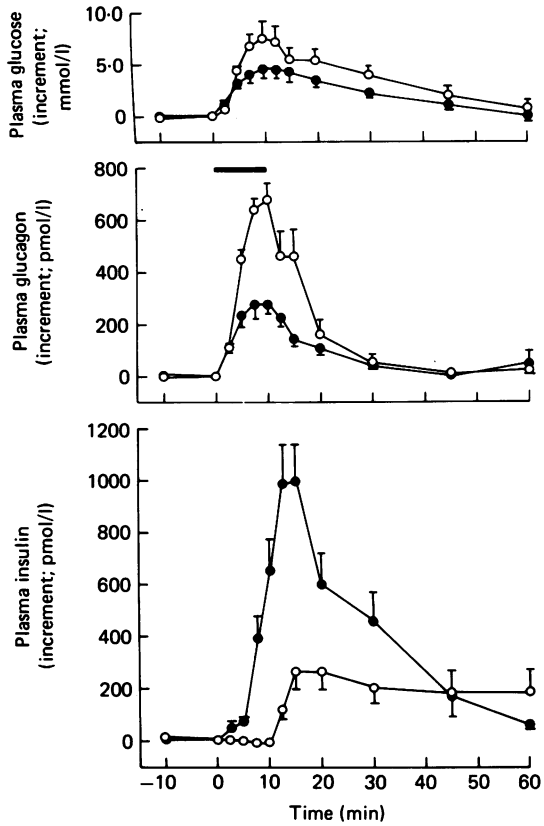

Fig. 1. Comparison of the changes in mean arterial plasma glucose, pancreatic glucagon and insulin concentrations in conscious 3 to 6-week-old adrenalectomized calves in response to stimulation of the peripheral ends of the splanchnic nerves at 40 Hz for 1 s at 10 s intervals for 10 min. ○, calves pre-treated with propranolol ($n = 8$); ●, calves pre-treated with phentolamine ($n = 8$). 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 calves pre-treated with propranolol: glucose, $3{\cdot}7 \pm 0{\cdot}3$ mmol/l; glucagon, 29 ± 10 pmol/l; insulin, 19 ± 5 pmol/l. In calves pre-treated with phentolamine: glucose, $3{\cdot}5 \pm 4$ mmol/l; glucagon, 14 ± 5 pmol/l; insulin, 44 ± 8 pmol/l.

similar to that in the control group (from 87 ± 3 to 142 ± 8 mmHg at 2·5 min, $P < 0{\cdot}01$; Fig. 4), presumably because the reflex bradycardia was thereby abolished; mean heart rate rose from 92 ± 5 to 96 ± 5 beats/min over this period (Fig. 4). However, mean blood pressure fell back towards the initial value much more rapidly in this than any other group. It is also noteworthy that pre-treatment with a large dose of hexamethonium (10 mg/kg) 10 min before stimulation failed to abolish the hypertensive response completely but there was no change in heart rate (Fig. 4).

The rise in haematocrit (from $38{\cdot}1 \pm 3{\cdot}1$ % to $44{\cdot}8 \pm 2{\cdot}6$ %) in calves which had been given all three post-ganglionic blocking agents was of roughly the same order of magnitude as that observed in all the groups recorded in Table 1, whereas there was no significant rise in calves given hexamethonium.

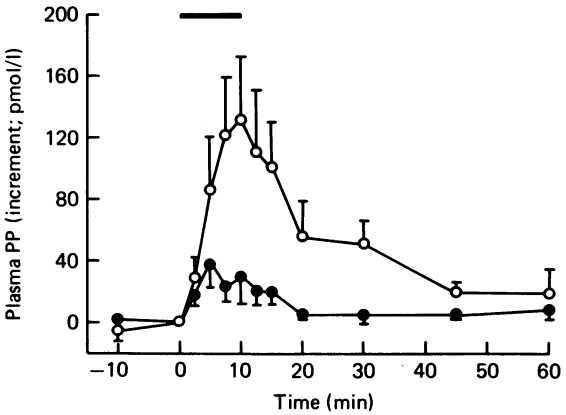

Fig. 2. Comparison of the changes in mean arterial plasma PP concentration in conscious 3 to 6-week-old adrenalectomized calves in response to stimulation of the peripheral ends of the splanchnic nerves at 40 Hz for 1 s at 10 s intervals for 10 min. ○, calves pre-treated with propranolol ($n = 8$); ●, calves pre-treated with phentolamine ($n = 8$). Horizontal bar, duration of stimulus. Vertical bars, S.E. of each mean value where these exceed the size of the symbol. Absolute value at time 0 in calves pre-treated with propranolol: 309 ± 3 pmol/l. In calves pre-treated with phentolamine: 38 ± 4 pmol/l.

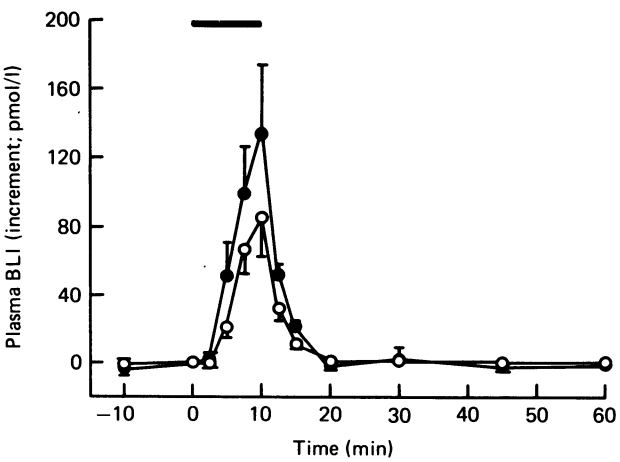

Fig. 3. Comparison of the changes in mean arterial plasma BLI in conscious 3 to 6-week-old adrenalectomized calves in response to stimulation of the peripheral ends of the splanchnic nerves at 40 Hz for 1 s at 10 s intervals for 10 min. ○, calves pre-treated with propranolol ($n = 8$); ●, calves pre-treated with phentolamine ($n = 8$). Horizontal bar, duration of stimulus. Vertical bars, S.E. of each mean value where these exceed the size of the symbol. Absolute value at time 0 in calves pre-treated with propranolol: 5 ± 2 pmol/l. In calves pre-treated with phentolamine: 9 ± 3 pmol/l.

Neuroendocrine responses. The pancreatic neuroendocrine responses to splanchnic nerve stimulation in bursts in calves pre-treated with both phentolamine and propranolol compared with the responses of normal control calves provided further substantive evidence that the adrenergic effects are due largely to activation of α-adrenoceptors (Figs. 1 and 5). These Figures show that the hyperglycaemic

response, the rise in mean plasma pancreatic glucagon and insulin concentrations are all closely similar in calves pre-treated with phentolamine to those in calves pre-treated with phentolamine and propranolol. The same responses were closely similar in the control group and the group pre-treated with propanolol alone.

The same pattern was observed in relation to the changes in mean arterial plasma PP concentration (Figs. 2 and 6). The response which occurred in the presence of phentolamine alone was closely similar to that recorded in calves pre-treated with both adrenergic blocking agents. The rise in mean arterial plasma PP was considerably greater in the control group (Fig. 6) than in the group pre-treated with propranolol but the peak differences (assessed both by the Student's t test and the Mann Whitney test) failed to achieve statistical significance and we are therefore disinclined to speculate about the involvement of β-adrenoceptors in this response.

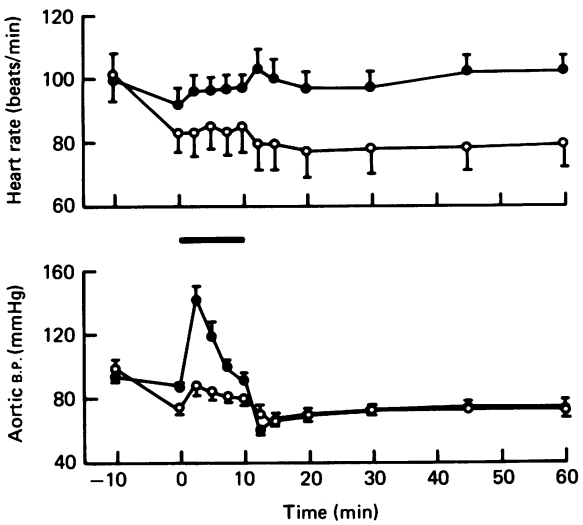

Fig. 4. Comparison of the changes in mean heart rate and aortic blood pressure in conscious 3 to 6-week-old adrenalectomized calves in response to stimulation of the peripheral ends of the splanchnic nerves at 40 Hz for 1 s at 10 s intervals for 10 min. ○, calves pre-treated with hexamethonium ($n = 6$); ●, calves pre-treated with propranolol, phentolamine and atropine ($n = 7$). Horizontal bar, duration of stimulus. Vertical bars, S.E. of each mean value.

The rise in mean arterial plasma BLI which occurred in response to intermittent splanchnic nerve stimulation was practically identical in control calves to that recorded in calves pre-treated with both propranolol and phentolamine (Fig. 7*A*). However, it is noteworthy that the initial mean value for the control group (7 ± 2 pmol/l) was significantly lower than that of the group pre-treated with both adrenergic blocking agents (25 ± 3 pmol/l, $P < 0{\cdot}01$).

The rise in mean arterial plasma BLI concentration in response to splanchnic nerve stimulation under precisely the same conditions was unaffected by additional pre-treatment with atropine and had risen by 190 ± 43 pmol/l at 10 min. In contrast, it was completely abolished by preganglionic blockade with hexamethonium. Pre-treatment with atropine in addition to both adrenergic post-ganglionic blocking

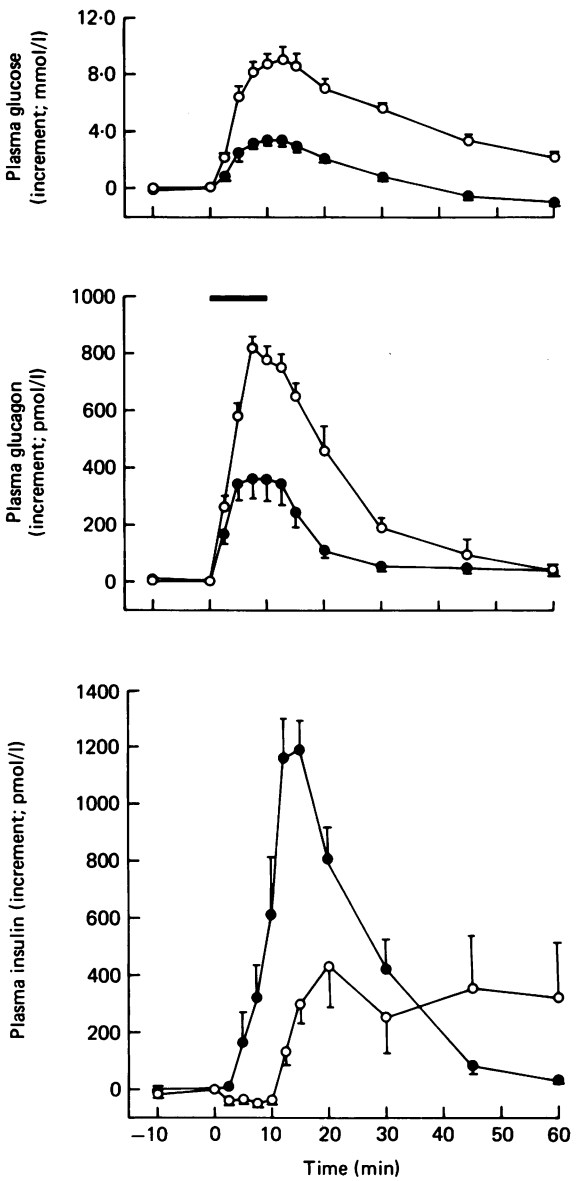

Fig. 5. Comparison of the changes in mean arterial plasma glucose, pancreatic glucagon and insulin concentration in conscious adrenalectomized 3 to 6-week-old calves in response to stimulation of the peripheral ends of the splanchnic nerves at 40 Hz for 1 s at 10 s intervals for 10 min. ○, control calves ($n = 6$); ●, calves pre-treated with propranolol and phentolamine ($n = 8$). Horizontal bar, duration of stimulus. Vertical bars, S.E. of each mean value. Absolute values at time 0 in the control group: glucose, $4{\cdot}3 \pm 0{\cdot}4$ mmol/l; glucagon, 35 ± 8 pmol/l; insulin, 66 ± 19 pmol/l. In the group pre-treated with propranolol and phentolamine: glucose, $2{\cdot}7 \pm 0{\cdot}5$ mmol/l; glucagon, 20 ± 6 pmol/l; insulin, 54 ± 13 pmol/l.

agents, significantly reduced the rise in mean arterial plasma insulin concentration. Thus, in the group given all three post-ganglionic blocking agents the mean incremental plasma insulin concentrations were 308 ± 104 pmol/l at 10 min, and 598 ± 214 pmol/l at the peak (12·5 min). The corresponding values in the absence of atropine were 1156 ± 143 and 1182 ± 107 pmol/l, producing P values of $< 0{\cdot}01$ and $< 0{\cdot}05$ respectively, using Student's t test (Figs. 5 and 8). None of the other pancreatic neuroendocrine responses, or the rise in mean plasma glucose concentration was significantly altered by additional pre-treatment with atropine (Figs. 5, 6 and 8). They were all effectively abolished by preganglionic blockade (Fig. 8).

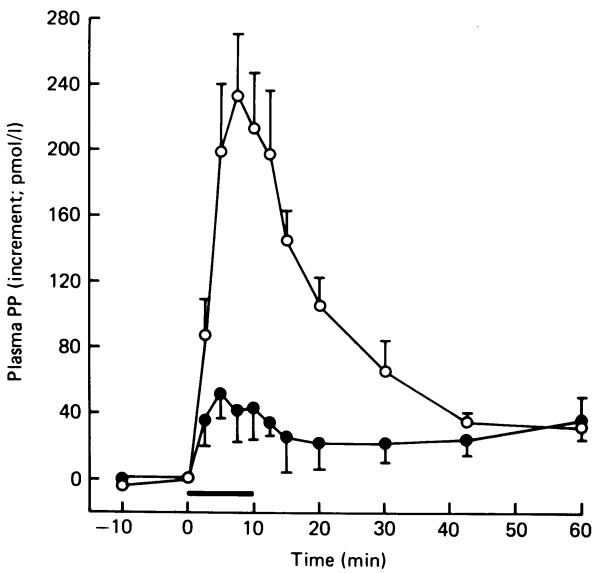

Fig. 6. Comparison of the changes in mean arterial plasma PP concentration of conscious 3 to 6-week-old calves in response to stimulation of the peripheral ends of the splanchnic nerves at 40 Hz for 1 s at 10 s intervals for 10 min. ○, control calves ($n = 6$); ●, calves pre-treated with propranolol and phentolamine ($n = 8$). Horizontal bar, duration of stimulus. Vertical bars, S.E.. of each mean value. Absolute values at time 0 in the control calves: 38 ± 9 pmol/l. In calves pre-treated with propranolol and phentolamine: 62 ± 10 pmol/l.

DISCUSSION

The results of these experiments show that the various neuroendocrine responses to splanchnic nerve stimulation that we have investigated in the conscious calf, are all abolished by preganglionic blockade. Under the same conditions, there was a small, transient rise in mean arterial blood pressure while both heart rate and haematocrit were unaffected. The mechanism of the hypertensive response is obscure. It might be due to release of a peptide such as substance P from afferent splanchnic nerve fibres stimulated antidromically. Alternatively, it is possible that there is a small population of post-ganglionic sympathetic fibres in the splanchnic nerves at the level at which the nerves were stimulated. The finding that there was a much more substantial (but transient) rise in mean aortic blood pressure in response to splanchnic nerve

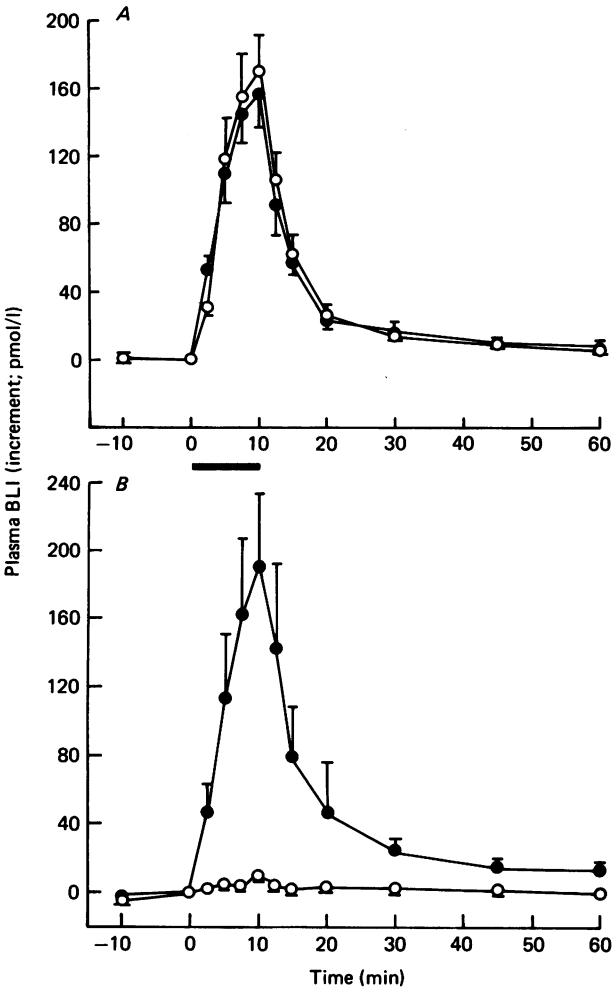

Fig. 7. Comparison of the changes in mean arterial plasma BLI of 3 to 6-week-old conscious adrenalectomized calves in response to stimulation of the peripheral ends of the splanchnic nerves at 40 Hz for 1 s at 10 s intervals for 10 min. *A* compares the mean response of normal control calves (○; $n = 6$) with that of calves pre-treated with propanolol and phentolamine (●; $n = 8$). The absolute value at time 0 in the control calves was 7 ± 2 pmol/l and in the other group was 25 ± 3 pmol/l. *B* compares the mean response of calves pre-treated with hexamethonium (○; $n = 6$) with that of calves pre-treated with propranolol, phentolamine and atropine (●; $n = 7$). The absolute value at time 0 in the group given hexamethonium was 27 ± 7 pmol/l and in the other was 44 ± 6 pmol/l. Horizontal bar, duration of stimulus. Vertical bars, S.E. of each mean value.

stimulation in the presence of atropine, propranolol and phentolamine was also unexpected, and suggests that there may be release of some vasoconstrictor peptide from post-ganglionic nerve terminals under these conditions. There was a significant rise in the concentration of PP in the arterial plasma in these experiments, and the finding that this peptide is capable of producing vascular effects (Lundberg & Tatemoto, 1982) raises the possibility that it may have contributed to the rise in blood pressure which occurred in these experiments.

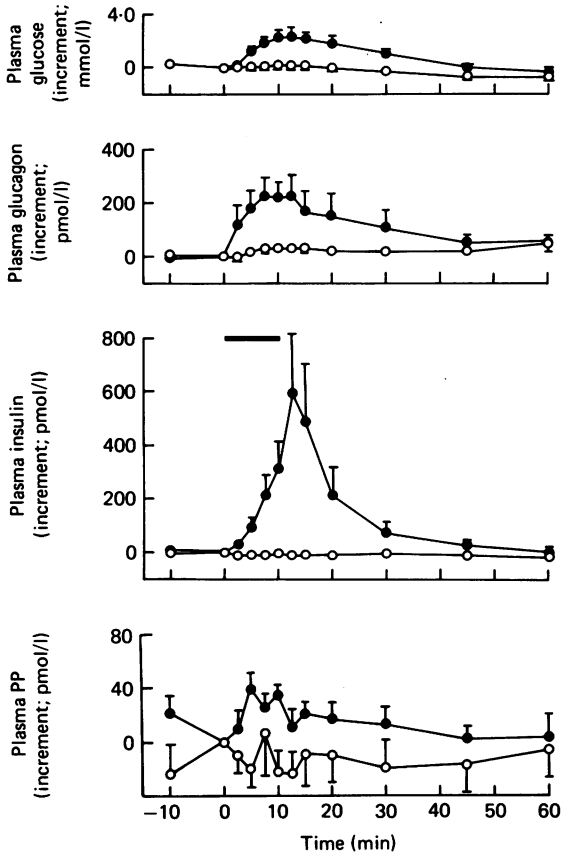

Fig. 8. Comparison of the changes in mean arterial plasma glucose, pancreatic glucagon, insulin and PP in conscious 3 to 6-week-old adrenalectomized calves in response to stimulation of the peripheral ends of the splanchnic nerves at 40 Hz for 1 s at 10 s intervals for 10 min. ○, calves pre-treated with hexamethonium ($n = 6$); ●, calves pre-treated with propranolol, phentolamine and atropine ($n = 7$). 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 pre-treated with the post-ganglionic blocking agents: glucose, 3·1 ± 0·3 mmol/l; glucagon, 30 ± 12 pmol/l; insulin, 35 ± 11 pmol/l; PP, 115 ± 22 pmol/l. In calves pre-treated with hexamethonium: glucose, 3·4 ± 0·3 mmol/l; glucagon, 33 ± 19 pmol/l; insulin, 30 ± 8 pmol/l; PP, 95 ± 21 pmol/l.

These results also show that none of the pancreatic neuroendocrine responses to stimulation of the sympathetic pancreatic innervation is entirely attributable to activation of adrenoceptors, although they are certainly implicated in various ways in the absence of blocking agents. Normally, the release of insulin is inhibited due to the overriding effect of the α-adrenoceptors. The same receptors can account for the adrenergic release of both glucagon and PP, but significant amounts of both these peptides are still released when the receptors have been blocked by phentolamine and there is then a massive release of insulin. This could not be accounted for by the rise in plasma glucose concentration because a comparable rise in plasma glucose, induced by intravenous infusions in conscious calves of the same age, produces a rise in plasma

insulin concentration of only about 200 pmol/l (Bloom & Edwards, 1981). None of these responses is attributable to β-adrenoceptors as they persist in the presence of propranolol at a high dose which sufficed to produce a significant fall in heart rate.

The further finding that the rise in mean arterial BLI was not diminished by total post-ganglionic adrenergic and cholinergic blockade, but was eliminated by pre-ganglionic blockade, indicates that the peptide is released from post-ganglionic sympathetic nerve terminals. It has been identified within nerve terminals in the gastrointestinal tract immunocytochemically and its distribution appears to be restricted to them (Dockray, Vaillant & Walsh, 1979). That contention is supported by the observation that there are significantly greater amounts of BLI in the smooth muscle of the gut (where the nerve terminals are) than the mucosa (Bloom, Edwards & Ghatei, 1983), and it has also been established that BLI is not released in response to vagal stimulation in conscious calves of the same age (Adrian, Bloom & Edwards, 1983). Whether it exerts an important effect as a transmitter when it is released from the post-ganglionic sympathetic nerve endings has yet to be established. It has been shown, however, that it is released in sufficient amounts to act in the classical hormonal way by transportation via the blood stream. The effects produced by intravenous infusions of synthetic amphibian bombesin, or the mammalian form of the molecule (GRP), include the release of pancreatic glucagon, insulin and PP. These responses occur when the two peptides are infused at a dose which reproduces the rise in mean arterial BLI which occurs in response to splanchnic nerve stimulation in bursts at 40 Hz (roughly 200 pmol/l), and it has been shown to be an exceptionally potent insulinotropic agent (Bloom *et al.* 1983). It therefore seems that all the pancreatic neuroendocrine responses to splanchnic nerve stimulation that occur in this species when both the α- and β-adrenoceptors have been blocked could be attributable largely to bombesin, which may be released either within the pancreas itself or from the gastrointestinal tract to be carried to the gland in the blood stream.

The observation that the release of insulin,which occurs when the α-adrenoceptors have been blocked, is significantly reduced by pre-treatment with atropine suggests the additional involvement of some muscarinic mechanism. Further work is necessary to elucidate this more precisely, but as the normal response to crude, total splanchnic nerve stimulation is inhibition of insulin release, its physiological importance is difficult to evaluate. Stimulation of the parasympathetic innervation to the gland in the conscious calf invariably provokes the release of insulin (Adrian *et al.* 1983) and it is certainly possible that there is a population of post-ganglionic muscarinic sympathetic neurones in the pancreas.

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