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PANCREATIC ENDOCRINE RESPONSES TO EXOGENOUS NEUROTENSIN IN THE CONSCIOUS CALF

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SUMMARY

1. Responses to neurotensin have been investigated in conscious calves 2–5 weeks after birth given continuous I.V. infusions of the peptide for 15 min (5 pmol . kg^{-1} . min^{-1}).

2. In control calves the concentration of the peptide in the arterial plasma had risen by 160 ± 10 pmol/l at the end of the infusion, after which it fell exponentially ($t_{\frac{1}{2}}$: 1·4 min).

3. This dose of neurotensin produced no significant change in mean heart rate, aortic blood pressure, plasma gastrin or glucose concentration.

4. It was found that neurotensin could produce a pronounced rise in the concentration of both insulin and pancreatic polypeptide (PP) in the arterial plasma, together with a much smaller rise in pancreatic glucagon concentration.

5. Each of these three pancreatic endocrine responses was found to be glucose-sensitive within the range *ca.* 5·0–9·0 mmol/l. Hyperglycaemia potentiated insulin release and inhibited release of PP and glucagon.

6. The results are discussed in relation to the findings of other workers in other species.

INTRODUCTION

Neurotensin has been found to cause hyperglycaemia, associated with hepatic glycogenolysis in the rat (Carraway, Demers & Leeman, 1973, 1976), and provokes a rise in plasma glucagon and fall in plasma insulin concentration in the same species (Brown & Vale, 1976). However, the doses employed in these experiments were very high (> 20 pmol/100 g body weight) and neurotensin is a potent vasodilator agent (Carraway & Leeman, 1973). Each of these hormonal and metabolic responses can readily be obtained by stimulating the splanchnic sympathetic innervation (see for instance Edwards & Silver, 1970; Edwards, 1972; Bloom, Edwards & Vaughan, 1973; Bloom & Edwards, 1975) and could therefore have arisen secondarily, as a consequence of a generalized increase in sympathetic efferent activity.

In the present experiments this question has been investigated by examining the pancreatic endocrine responses to infusions of exogenous bovine neurotensin in conscious calves at a dose below that which produces any discernible change in aortic

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blood pressure of heart rate (5 pmol.kg^{-1}.min^{-1}). Under these conditions neurotensin exerts a weak stimulatory effect on the release of pancreatic glucagon and strongly stimulates the release of both insulin and pancreatic polypeptide (PP) from the pancreas. Each of these effects is glucose-dependent over the range 5–9 mmol/l.

Certain of these results have been published previously in a preliminary form (Blackburn, Edwards, Adrian & Bloom, 1981).

METHODS

Animals

The experiments were carried out on pedigree Jersey and Jersey × Charollais calves which were obtained from local farms shortly after birth and used at ages ranging between 17 and 39 days (24·4–37·5 kg body weight). The animals were kept in individual pens in the laboratory animal house and maintained on a diet of milk (6–7 pints/day). Food was withheld for at least 6 h prior to surgery and for at least 14 h before each experiment.

Experimental procedures

Preparatory surgery involved the insertion of narrow-bore 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 collection of arterial samples. In addition a similar catheter was inserted into one or both saphenous veins to provide a conduit for I.V. infusions or injections. These procedures were carried out under general anaesthesia, induced with chloroform and maintained with halothane. Procaine penicillin (600,000 i.u.) and dihydrostreptomycin (0·5 g) were administered routinely.

Experiments were carred out at least 24 h after surgery and were invariably started at the same time of day in order to minimize any diurnal variations. Synthetic bovine neurotensin (Bachem Inc., California) was dissolved in *ca.* 20 ml 2% (v/v) calf plasma in sterile 0·9% saline (w/v) containing 2,000 K.I.U. aprotinin/ml (Trasylol: Bayer); the final volume was adjusted in such a way that infusion of this mixture at a rate of 1 ml/min resulted in delivery of neurotensin at a dose of 5 pmol.kg^{-1}.min^{-1}. Immediately before and after each experimental infusion of the peptide a timed sample of the infusate was collected into a known volume of calf plasma, containing aprotinin, for subsequent radioimmunoassay, in order to check the validity of the dose of neurotensin infused. Calves given exogenous glucose received a continuous I.V. infusion (0·03 mmol.kg^{-1}.min^{-1}) which was initiated 90 min before neurotensin was administered. 'Six times recrystallized' bovine insulin (Boots Pure Drug Co. Ltd) was dissolved in acidulated sterile saline and injected I.V. at a dose of 0·7 nmol/kg body weight when required.

Heart rate and aortic blood pressure were monitored continuously throughout each experiment, by means of a Devices L221 pressure transducer connected to a Devices M19 pen recorder.

Estimations

Samples of arterial blood were collected at intervals for peptide and glucose measurements. Aliquots destined for peptide assays were collected into heparinized tubes containing aprotinin (2,000 K.I.U./ml blood) and centrifuged without delay at 4 °C; the plasma was subsequently stored at −20 °C. Glucose was measured by means of a Mark 2 Beckman Glucose Analyser. Pancreatic glucagon was measured by a radioimmunoassay using an antiserum relatively specific for pancreatic glucagon which was C-terminal-reacting (Assan & Slusher, 1972) and reacted less than 5% with 'glucagon-like immunoreactivity of ileal origin' (enteroglucagon). Insulin, PP, neurotensin and gastrin (G17 and G34) were also measured by radioimmunoassay (Albano, Ekins & Turner, 1972; Adrian, Bloom, Bryant, Polak, Heitz & Barnes, 1977; Blackburn & Bloom, 1979; Russell, Bloom, Fielding & Bryant, 1976).

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

RESULTS

Effects of infusions of neurotensin in normal calves

Intravenous infusions of neurotensin, at a dose of 5 pmol . kg . min^{-1} for 15 min, in six 2–5-week-old calves produced a steady rise in the mean concentration of the peptide in the arterial plasma, from an initial value of 29·5 ± 3·6 pmol/l, to achieve an incremental plateau between 12½ and 15 min (160 ± 10 pmol/l at 15 min; Fig. 1*A*). When the infusion was discontinued the mean incremental plasma neurotensin concentration fell exponentially (Fig. 1*B*; $r = 0{\cdot}97$) with a half-life of 1·4 min and

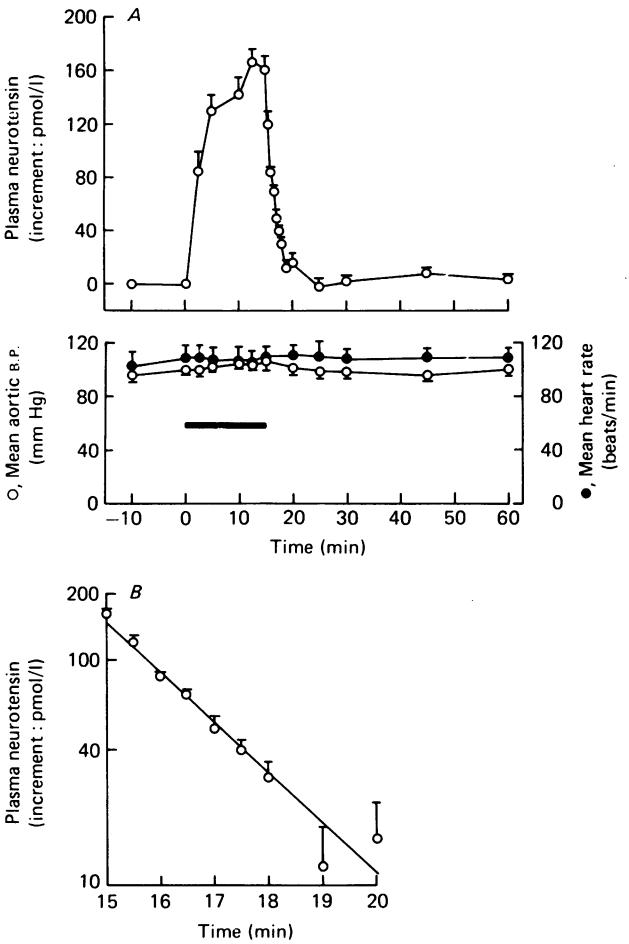

Fig. 1. *A*, changes in mean arterial plasma neurotensin concentration, heart rate and aortic blood pressure in response to I.V. infusion of exogenous neurotensin (5 pmol . kg^{-1} . min^{-1} for 15 min) in normal control 2–5-week-old calves ($n = 6$). Horizontal bar: duration of infusion. Vertical bars: S.E. of each mean value. Absolute neurotensin concentration at time 0: 29·5 ± 3·6 pmol/l. *B*, change in mean arterial plasma neurotensin concentration during the 5 min immediately after infusion was discontinued, using expanded semi-log scale. $t_{\frac{1}{2}} = 1{\cdot}4$ min; $r = 0{\cdot}9714$.

had returned to within the initial range at 25 min (Fig. 1*A*). No significant change in heart rate or aortic blood pressure was detected at any stage during these experiments (Fig. 1*A*) and the infusions failed to produce any behavioural response in these conscious calves.

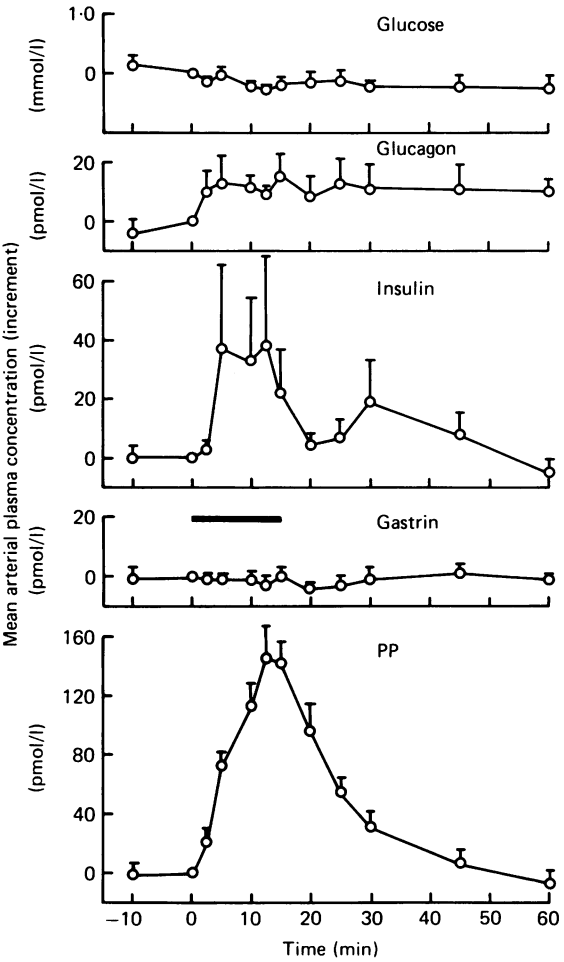

Fig. 2. Changes in mean arterial plasma glucose, pancreatic glucagon, insulin, gastrin and PP concentration in response to I.V. infusion of exogenous neurotensin (5 pmol . kg^{-1} . min^{-1} for 15 min) in normal control 2–5-week-old calves ($n = 6$). Horizontal bar: duration of infusion. Vertical bars: S.E. of each mean value. Absolute values at time 0: glucose, 5·04 ± 0·39 mmol/l; pancreatic glucagon, 26 ± 13 pmol/l; insulin, 13·3 ± 3·3 pmol/l; gastrin, 15·8 ± 3·3 pmol/l; PP, 38·8 ± 7·4 pmol/l.

Neurotensin produced a small but rapid rise in the mean arterial plasma concentrations of both pancreatic glucagon and insulin. Neither response achieved statistical significance in this small group of animals and there was no significant change in arterial plasma glucose concentration (Fig. 2). In contrast there was a rapid and substantial rise in mean arterial plasma PP concentration, which rose from an initial

value of 38·8 ± 7·4 pmol/l to a peak incremental value of 145 ± 21 pmol/l at 12½ min ($P < 0.001$). Mean plasma gastrin concentration was apparently unaffected by neurotensin under these conditions (Fig. 2).

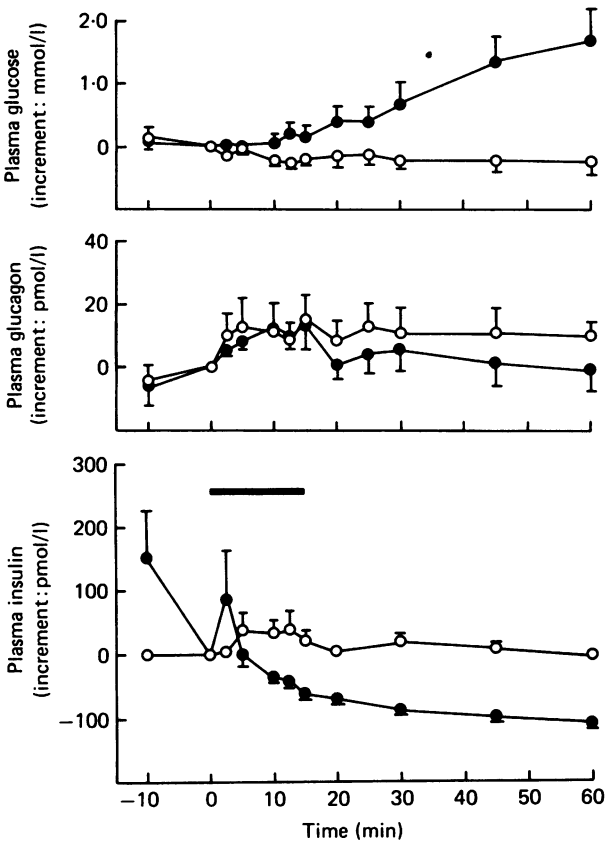

Fig. 3. Comparison of the changes in mean arterial plasma glucose, pancreatic glucagon and insulin concentration in response to I.V. infusion of neurotensin (5 pmol . kg^{-1} . min^{-1} for 15 min) in normal control 2–5-week-old calves (open circles; $n = 6$) and 2–5-week-old calves given insulin (0·7 nmol/kg I.V.) 40 min previously (filled circles; $n = 7$). Horizontal bar: duration of infusion. Vertical bars: S.E. of each mean value where these exceed the size of the symbol. Absolute values at time 0 in control group: glucose, 5·04 ± 0·39 mmol/l; pancreatic glucagon, 26 ± 13 pmol/l; insulin, 13·3 ± 3·3 pmol/l. Experimental group: glucose, 3·00 ± 0·21 mmol/l; pancreatic glucagon, 20·2 ± 4·9 pmol/l; insulin, 148·6 ± 9·4 pmol/l.

The further question, whether the pancreatic endocrine responses to neurotensin were glucose-sensitive, was investigated by infusing neurotensin in the same way, in calves in which the initial arterial plasma glucose concentration had been lowered by prior administration of insulin or raised by infusing glucose.

Effects of infusions of neurotensin during moderate hypoglycaemia

In these experiments the animals were given a small dose of exogenous insulin (0·7 nmol/kg body weight) by rapid I.V. injection 40 min before infusion of neurotensin. This protocol was adopted in the hope of examining the responses to neurotensin during moderate hypoglycaemia at a time when the plasma insulin concentration had returned to within the physiological range. Neurotensin was infused at the same dose

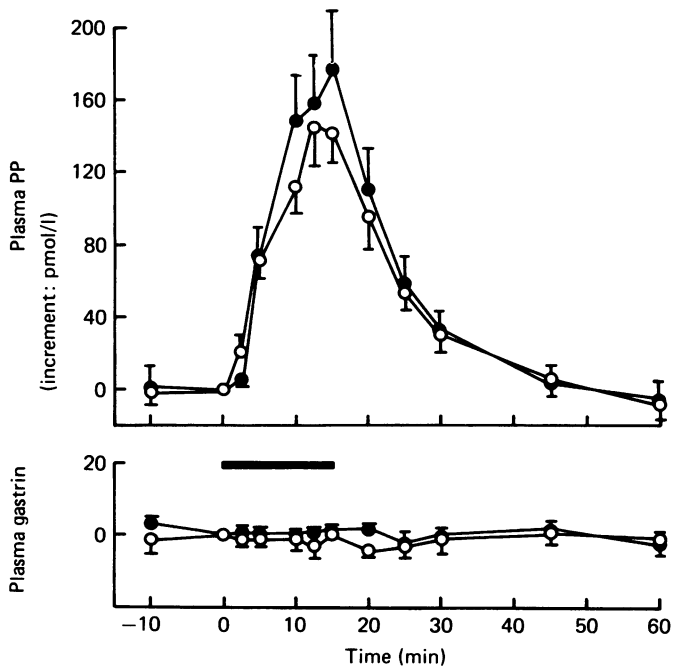

Fig. 4. Comparison of the changes in mean arterial plasma PP and gastrin concentration in response to I.V. infusion of exogenous neurotensin (5 pmol . kg^{-1} . min^{-1} for 15 min) in normal control 2–5-week-old calves (open circles; $n = 6$) and 2–5-week-old calves given insulin (0·7 nmol/kg I.V.) 40 min previously (filled circles; $n = 7$). Horizontal bar: duration of infusion. Vertical bars: S.E. of each mean value. Absolute values at time 0 in control group: glucose, 5·04 ± 0·39 mmol/l; PP, 38·8 ± 7·4 pmol/l; gastrin, 15·8 ± 3·3 pmol/l. Experimental group: glucose, 3·00 ± 0·21 mmol/l; PP, 42·7 ± 13·2 pmol/l; gastrin, 10·6 ± 2·8 pmol/l.

and for the same period as had been employed in the control group (5 pmol . kg^{-1} . min^{-1} for 15 min). The mean arterial plasma glucose and insulin concentrations in these animals immediately prior to infusion of neurotensin were 3·00 ± 0·21 mmol/l and 148·6 ± 9·4 pmol/l respectively, compared with control values of 5·04 ± 0·39 mmol/l and 13·3 ± 3·3 pmol/l. No significant difference was observed between the changes in mean arterial plasma glucose and glucagon concentration during the infusion of neurotensin (Fig. 3), although the plasma glucose concentration rose steadily thereafter in the experimental group, as the animals recovered from the insulin hypoglycaemia that had been induced 40 min before infusion. Interpretation of the

changes in mean plasma insulin concentration are complicated by the fact that it was falling steadily (approximately exponentially) throughout each of these experiments, presumably from a peak immediately after the priming I.V. dose of exogenous hormone (Fig. 3). Even so, the fact that a small rise in plasma insulin was

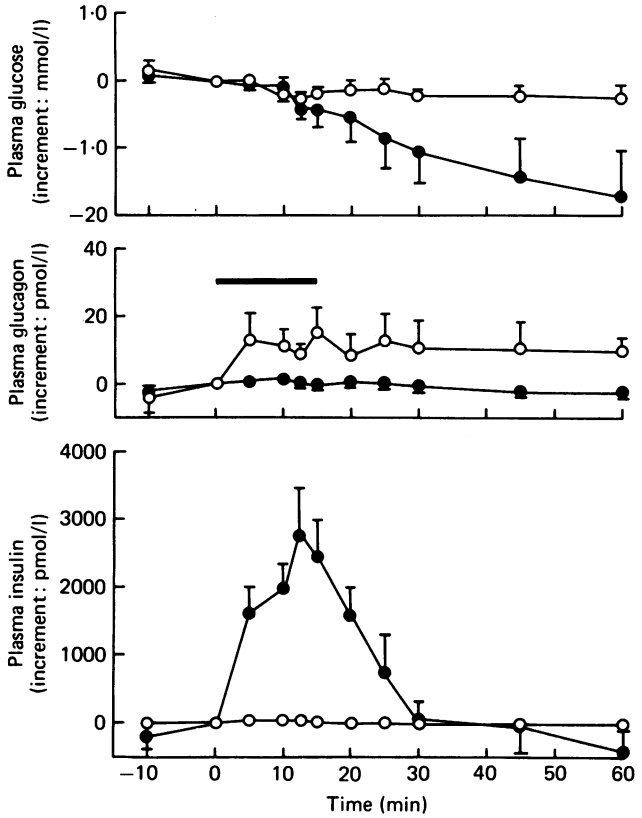

Fig. 5. Comparison of the changes in mean arterial plasma glucose, pancreatic glucagon and insulin concentration in response to I.V. infusion of exogenous neurotensin (5 pmol . kg^{-1} . min^{-1} for 15 min) in normal control 2–5-week-old calves (open circles; $n = 6$) and 2–5-week-old calves receiving a continuous I.V. infusion of glucose (0·03 mmol . kg^{-1} . min^{-1}; filled circles; $n = 8$). Horizontal bar: duration of neurotensin infusion. Vertical bars: S.E. of each mean value where these exceed the size of the symbol. Absolute values at time 0 in control group: glucose, 5·04 ± 0·39 mmol/l; pancreatic glucagon, 26 ± 13 pmol/l; insulin, 13·3 ± 3·3 pmol/l. Experimental group: glucose, 9·49 ± 0·50 mmol/l; pancreatic glucagon, 7 ± 2 pmol/l; insulin, 1255 ± 272 pmol/l.

superimposed on this falling pattern of values within 2½ min, in response to neurotensin, suggests that the 'insulin response' is not completely suppressed in all animals by this degree of hypoglycaemia. No attempt has been made to correct these data for the distortion produced by the initial injection of exogenous insulin.

The rise in mean arterial plasma PP concentration in response to neurotensin was closely similar to that in the control group, showing that this pancreatic endocrine response, like the release of glucagon from the pancreas, is unaffected by reducing

plasma glucose concentration within the range *ca.* 5·0–3·0 mmol/l (Fig. 4). It was also found that this dose of neurotensin caused no rise in mean plasma gastrin concentration during hypoglycaemia so moderate as to cause no gastrin response *per se* (Fig. 4).

Effects of infusions of neurotensin during hyperglycaemia

In these experiments the animals received a continuous I.V. infusion of glucose (0·03 mmol.kg^{-1}.min^{-1}). The effect of this manoeuvre was to raise initial mean arterial plasma glucose concentration by *ca.* 4·5 mmol/l.

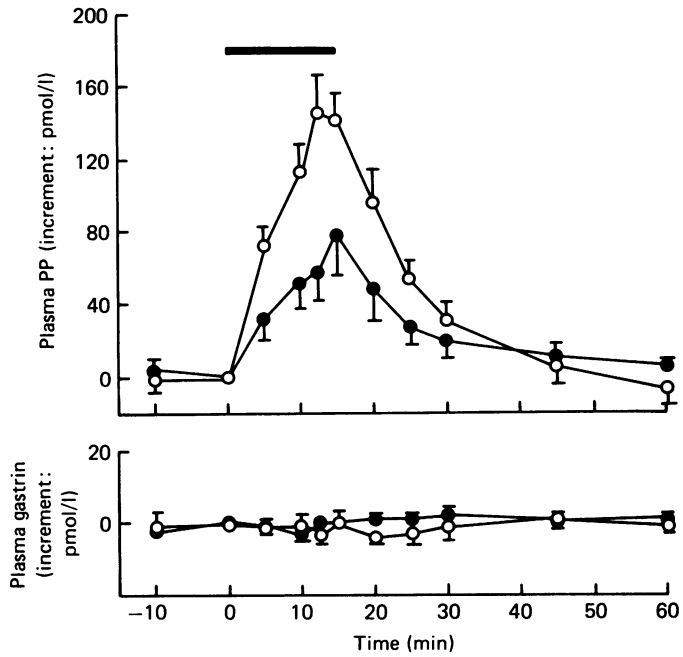

Fig. 6. Comparison of the changes in mean arterial plasma PP and gastrin concentration in response to I.V. infusion of exogenous neurotensin (5 pmol.kg^{-1}.min^{-1} for 15 min) in normal control 2–5-week-old calves (open circles; $n = 6$) and 2–5-week-old calves receiving a continuous I.V. infusion of glucose (0·03 mmol.kg^{-1}.min^{-1}; filled circles; $n = 6$). Horizontal bar: duration of neurotensin infusion. Vertical bars: S.E. of each mean value. Absolute values at time 0 in control group: glucose, 5·04±0·39 mmol/l; PP, 38·8±7·4 pmol/l; gastrin, 15·8±3·3 pmol/l. Experimental group: glucose, 9·49±0·50 mmol/l; PP, 13·0±2·1 pmol/l; gastrin, 10·6±2·8 pmol/l.

Under these hyperglycaemic conditions the rise in plasma glucagon concentration in response to neurotensin was completely suppressed and, even though the glucagon response in the control group was quite small, the difference between the two mean incremental plasma concentrations achieved statistical significance at 12½ min ($P < 0·05$; Fig. 5). In contrast, release of insulin in response to neurotensin was massively potentiated by hyperglycaemia. The peak incremental mean plasma insulin concentration in the control group at 12½ min was 38·2±29·8 pmol/l compared with a corresponding value of 2772±714 pmol/l in the experimental group ($P < 0·01$).

This occurred in spite of the fact that the basal plasma insulin concentration immediately before the infusion of neurotensin was significantly higher in the experimental (1255 ± 272 pmol/l) than the control group ($13{\cdot}3 \pm 3{\cdot}3$ pmol/l; $P < 0{\cdot}01$), having already risen in response to the glucose infusion. It is also noteworthy that the release of insulin in the experimental group produced a perceptible fall in mean plasma glucose concentration in spite of the continued infusion of glucose (Fig. 5).

Mean arterial plasma gastrin concentration was not significantly affected either by the infusion of glucose or the superimposed infusion of neurotensin (Fig. 6). In direct contrast, infusion of glucose produced a significant reduction both in basal PP concentration and the incremental rise in response to neurotensin. Thus the mean arterial plasma PP concentration just before neurotensin was infused was $38{\cdot}8 \pm 7{\cdot}4$ pmol/l in the control and $13{\cdot}0 \pm 2{\cdot}1$ pmol/l in the experimental group ($P < 0{\cdot}01$). The peak mean incremental value at $12\frac{1}{2}$ min was 145 ± 21 pmol/l in the control group and 58 ± 16 pmol/l in the experimental group ($P < 0{\cdot}01$).

DISCUSSION

The results of these experiments are in agreement with those of Ukai and Kaneto and their collaborators, who concluded that neurotensin is capable of stimulating the release of glucagon and insulin from the pancreas (Ukai, Inoue & Itatsu, 1977; Kaneto, Kaneko, Kajinuma & Kosaka, 1978). Under the conditions of our experiments the effect on glucagon release was rather weak but, in view of the extreme sensitivity to glucose of the insulin response to neurotensin, it is at least possible that different experimental conditions might be found to potentiate this action of neurotensin as well. The pronounced glucose-sensitivity of the insulin response to neurotensin provides a possible explanation for the conflicting findings of Brown & Vale (1976), as the absolute plasma glucose concentration in their experiments was not recorded. However, in view of the fact that the minimum dose they found to be effective (*ca.* 7,000 pmol/kg body weight) far exceeds the threshold dose that will produce vasodilatation and consequent hypotension under the conditions of their experiments (100 pmol/kg: Carraway & Leeman, 1973), it seems much more likely that the inhibition of insulin release is attributable to a secondary sympathetic discharge. The same explanation would account for the finding that neurotensin causes hyperglycaemia and hepatic glycogenolysis (Carraway *et al.* 1973, 1976), as the dose they found to be minimally effective (300 pmol/kg) was well within the hypotensive range and the hyperglycaemic response that they described was linearly related to the dose of neurotensin between 300 and 2,000 pmol/kg. The grounds on which these authors exclude the possibility that the hyperglycaemic and glycogenolytic responses they observed were secondary to sympathetic activity (persistence following adrenergic blockade or depletion) are invalid. Both the release of glucagon from the pancreas and the mobilization of liver glycogen that occur so readily in response to stimulation of the sympathetic innervation have been shown to persist in the presence of adrenergic blocking agents (Shimazu & Amakawa, 1968; Bloom & Edwards, 1978) and may well be mediated by some non-adrenergic transmitter (Edwards & Bloom, 1978).

In the present experiments infusions of neurotensin at a dose (5 pmol.kg^{-1}.min^{-1})

twenty times lower than that which causes hypotension in the rat, and which had no significant effect on either heart rate or blood pressure in the conscious calf, raised the concentration of the peptide in the circulating plasma by about 160 pmol/l. This compares with rises of 58 pmol/l following ingestion of fat (Rosell & Rökaeus, 1979) and of 27 ± 8 pmol/l after a normal meal in man (Blackburn & Bloom, 1979). The absence of any significant rise in plasma glucose concentration under these conditions therefore provides strong evidence that the peptide does not act directly on the liver to produce glycogenolysis at a concentration compatible with hormonal action *in vivo*.

Release of PP and insulin from the pancreas in the presence of optimal glucose concentrations appear to be among the most sensitive responses to neurotensin that have so far been described. However, it still remains to be ascertained whether these effects are produced in response to changes in the concentration of neurotensin in the plasma that occur under normal physiological conditions. The importance of satisfying this criterion in respect of any action this peptide may be shown to have, before ascribing it a hormonal function, is underlined by the finding that it is eliminated from the circulating plasma so rapidly ($t_{\frac{1}{2}}$: 1·4 min).

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