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Effects of intravenous neuropeptide Y on insulin secretion and insulin sensitivity in skeletal muscle in normal rats

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Summary Intracerebroventricular administration of neuropeptide Y to normal rats induces a syndrome characterised by obesity, hyperinsulinaemia, insulin resistance and over expression of the adipose tissue *ob* gene. Little is known about the effect of circulating neuropeptide Y on glucose metabolism, insulin secretion and leptin. We therefore aimed to evaluate the effect of an intravenous infusion of neuropeptide Y on glucose disposal, endogenous glucose production, whole body glycolytic flux, and glucose storage as assessed during euglycaemic hyperinsulinaemic clamp. In addition, the insulin-stimulated glucose utilisation index in individual tissues was measured by the 2-deoxy-[1-^{3}H]-glucose technique. The effect of neuropeptide Y on insulin secretion was evaluated by hyperglycaemic clamp. Infusion did not induce any change in endogenous glucose production during basal conditions or at the end of the clamp. Glucose disposal was significantly increased in the rats given neuropeptide Y compared with controls (27.8 ± 1.3 vs 24.3 ± 1.6 mg · min^{-1} · kg^{-1}; $p < 0.05$) as was the glycolytic flux (18.9 ± 1.6 vs 14.4 ± 0.8 mg · min^{-1} · kg^{-1}; $p < 0.05$), while glucose storage was comparable in the two groups. In skeletal muscle, the glucose utilisation index was increased significantly in rats given neuropeptide Y. The glucose utilisation index in subcutaneous and epididimal adipose tissue was not significantly different between the two groups. Plasma leptin was significantly increased by hyperinsulinaemia, but was not affected by neuropeptide Y infusion. Both the early and late phase of the insulin response to hyperglycaemia were significantly reduced by neuropeptide Y. In conclusion neuropeptide Y infusion may increase insulin-induced glucose disposal in normal rats, accelerating its utilisation through the glycolytic pathway. Neuropeptide Y reduces both phases of the insulin response to hyperglycaemia. [Diabetologia (1998) 41: 1361–1367]

Keywords Neuropeptide Y, insulin secretion, insulin sensitivity, leptin, clamp technique, rat

Neuropeptide Y is a potent stimulator of food intake [1–4]. It is widely distributed in the central nervous system, but at the hypothalamic level it is synthesised in the arcuate nucleus, from where it is released in the paraventricular nucleus [5]. Previous studies have shown that chronic intracerebroventricular administration of neuropeptide Y in normal rats increases food intake and produces a syndrome characterised by obesity, hyperinsulinaemia, increased metabolic activity of white adipose tissue and muscle insulin resistance. Several of these metabolic effects are still present when increased food intake is prevented by food restriction [3]. Hence, it is probable that neuropeptide Y is a major neuromodulator with a role in nutrient partitioning. Moreover the obesity syndrome produced by intracerebroventricular neuropeptide Y is characterised by increased expression of the *ob* gene in adipose tissue [6]. On the other hand the *ob* gene product, leptin, has been shown to inhibit neuropeptide Y synthesis and release from hypothalamic nuclei in ob/ob mice [7]. Thus, the existence of a long loop control system in

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energy metabolism has been identified between the brain and adipose tissue.

Neuropeptide Y is also present in circulating blood, where it comes mainly from the spillover of the sympathetic nerve terminals and from the adrenal medulla [8]. Its plasma concentrations rise in response to muscular exercise [9] and in disorders such as pheochromocytoma and renal failure [10]. Neuropeptide Y is present in the pancreas, in both the islet cells and in sympathetic nerve terminals [11, 12]. However, whether peripheral neuropeptide Y has a hormone-like action and directly influences glucose metabolism and/or insulin secretion in vivo remains unclear. It should be noted in this respect that neuropeptide Y, at high concentrations, may contribute to the modulation of insulin secretion in vitro [13, 14]. It has also been reported that neuropeptide Y may reduce plasma glucose concentrations during exercise by inhibiting glycogen breakdown in the splanchnic compartment [15, 16]. Moreover, the potential relation between circulating neuropeptide Y and the pathophysiological consequences of obesity are far from clear.

This study aimed to evaluate the effects of short term intravenous neuropeptide Y infusion on the overall glucose disposal in vivo during euglycaemic hyperinsulinaemic clamp and to assess glucose utilisation in different tissues, using clamps associated with the labelled 2-deoxyglucose technique. The impact of neuropeptide Y on in vivo insulin secretion was evaluated during hyperglycaemic clamps. The influence of neuropeptide Y on serum leptin values was also estimated during euglycaemic hyperinsulinaemic clamp.

Materials and methods

Animals. Twelve week old male Sprague Dawley rats were used throughout the study. Animals were housed in our animal quarters under conditions of controlled temperature (23 °C) and light (6 am–6 pm), and had free access to water and a standard laboratory chow (Zoopharma, Padua, Italy). The protocol was approved by an internal ethics committee and experiments were performed in agreement with the rules of the laboratory animal care and the Italian law on animal experimentation.

Surgical procedure. After an overnight fast, rats were anaesthetised by intraperitoneal injection of pentobarbital (50 mg/kg). Two indwelling catheters were inserted – one in the right jugular vein for infusion of glucose, insulin and tracers and the other in the left carotid artery for blood sampling, as previously described in details [17]. Body temperature was maintained at 37 °C throughout the study by means of a heating blanket connected to a rectal probe.

Euglycaemic-hyperinsulinaemic clamp and glucose turnover rate. To evaluate the effects of the peripheral neuropeptide Y infusion on the glucose disappearance rate, endogenous glucose production and glycolytic flux in 12 rats, a 30 min recovery period from surgery was followed by a primed-continuous 3-^{3}H-glucose infusion (0.15 μCi · min^{-1}) to reach a steady state of glucose tracer after 30 min [18]. In six rats, a primed-continuous infusion of neuropeptide Y (60 pmol · min^{-1} · kg^{-1}) was then started and was continued throughout the study to reach a stable increase in plasma neuropeptide Y concentrations. Starting 30 min after the neuropeptide Y infusion (time 0), a human insulin solution (Actrapid, Novo, Bagsvaerd, Denmark) was infused at a rate of 15 mIU · min^{-1} · kg^{-1} together with a 20 % glucose solution, to maintain the arterial plasma glucose concentrations at the euglycaemic values.

Arterial blood samples (200 μl) were collected before the tracer infusion (at time –60 min), 30 min after neuropeptide Y infusion (time 0 min) and at the end of the clamp (time 120 min) to measure glucose, insulin and lactate concentrations. Small aliquots (30 μl) of blood were taken every 5–10 min during the clamp for the measurement of plasma glucose. In addition, samples (50 μl) were collected at times –30, 0 and every 10 min during the last 60 min of the clamp, by which time a constant glucose infusion rate and plasma glucose specific activity had been reached for determination of plasma radioactivity. Samples of arterial blood (50 μl) were precipitated in 250 μl $ZnSO_4$ and 250 μl $Ba(OH)_2$ and centrifuged immediately. Aliquots of the supernatant were used to measure the glucose concentration and 3-^{3}H-glucose radioactivity. Glucose turnover and endogenous glucose production were calculated in the basal state, after 30 min of neuropeptide Y infusion and in the insulin-stimulated state, as described previously [17]. Glycolytic flux and glucose storage were calculated as described by Rossetti and Giaccari [19] and reported previously by our laboratory [18].

Measurement of tissue glucose utilisation index. Two groups of six rats were used to assess the effect of peripheral neuropeptide Y infusion on glucose utilisation by individual tissues. A primed continuous infusion of neuropeptide Y (60 pmol · min^{-1} · kg^{-1}) or saline was started at the end of the recovery period and continued throughout the experiment. After 30 min (time 0), insulin and a glucose solution were infused as described above to reach a new euglyaemic-hyperinsulinaemic steady state. When the latter was reached (after about 70–90 min), a bolus of 2-deoxy-[1-^{3}H]-glucose (2DG, 30 μCi) was injected through the jugular vein and arterial samples (50 μl) were collected for blood 2-deoxyglucose specific activity at times 1, 3, 5, 10, 20, 30 min after the 2DG injection. Aliquots of arterial plasma were collected at times –30 and 0 min, and at the end of clamp for determination of plasma insulin, glucose, non-esterified fatty acid, lactate, and neuropeptide Y concentrations. Thirty minutes after the 2DG injection, the animals were killed and tissues were collected for the determination of the 2DG and 2DG6P content. The following tissues were collected: diaphragm, white and red quadriceps, white and red gastrocnemius, epidydimal and subcutaneous adipose tissue. The 2DG and 2DG6P contents were measured as described previously [20].

Hyperglycemic clamp. To study the effect of neuropeptide Y infusion on insulin secretion, hyperglycaemic clamp studies were carried out as previously described in rats [17]. The surgical procedure was the same as above. After 30 min of recovery from surgery, an infusion of saline or neuropeptide Y (60 pmol · min^{-1} · kg^{-1}) was started in two groups of six animals and continued throughout the experiments. After 30 min (time 0 min), a 20 % glucose solution was infused at a rate of 70, 57, 46, 35 and 25 mg · min^{-1} · kg^{-1} at times 0, 1, 2, 3, 4 and 5 min respectively. The glucose infusion rate was monitored via plasma glucose concentrations to obtain clamps at 13–13.5 mmol/l (coefficient of variance < 10 %). Blood samples (30 μl) for glucose assay were collected at times –30 and

Table 1. Biochemical and hormonal profile of control rats ($n = 6$) and rats given neuropeptide Y infusion ($n = 6$) in basal conditions, after 30 min of saline or neuropeptide Y infusion and at the end of euglycaemic hyperinsulinaemic clamp

Time/ Group	Leptin ($ng \cdot ml^{-1}$)	Neuropeptide Y ($ng \cdot ml^{-1}$)	Glucose ($mmol \cdot l^{-1}$)	Insulin ($\mu UI \cdot ml^{-1}$)	Lactate ($mmol \cdot l^{-1}$)	Non-esterified fatty acid ($\mu mol \cdot l^{-1}$)
Baseline						
Control	0.49 ± 0.11	2.8 ± 0.3	6.1 ± 0.3	24 ± 12	0.95 ± 0.21	792 ± 76
neuropeptide Y	0.63 ± 0.08	2.6 ± 0.8	6.4 ± 0.2	30 ± 10	0.80 ± 0.10	629 ± 62
30 min after saline or neuropeptide Y infusion						
Control	0.58 ± 0.16	2.3 ± 0.4	5.5 ± 0.4	30 ± 10	1.02 ± 0.08	825 ± 77
neuropeptide Y	0.69 ± 0.10	7.1 ± 1.3[a]	6.3 ± 0.3	28 ± 8	0.89 ± 0.05	731 ± 41
End of clamp						
Control	1.15 ± 0.10[b]	2.5 ± 0.8	6.1 ± 0.2	121 ± 16[b]	1.47 ± 0.09[b]	269 ± 39
neuropeptide Y	1.41 ± 0.22[b]	10.1 ± 1.8[a,b]	6.1 ± 0.2	137 ± 9[b]	1.09 ± 0.06[a]	260 ± 41

Mean ± SE
Statistical analysis performed by ANOVA.
[a] $p \leq 0.05$ compared with control group; [b] $p \leq 0.05$ compared with baseline value

Table 2. Parameters of glucose metabolism assessed during basal conditions, after 30 min of saline ($n = 6$) or neuropeptide Y ($n = 6$) infusion and at the end of euglycaemic hyperinsulinaemic clamp

Time/group	Endogenous glucose production ($mg \cdot kg^{-1} \cdot min^{-1}$)	Glucose disappearance rate (M)* ($mg \cdot kg^{-1} \cdot min^{-1}$)	Glycolytic flux ($mg \cdot kg^{-1} \cdot min^{-1}$)	Glucose storage ($mg \cdot kg^{-1} \cdot min^{-1}$)
Basal				
Control	12.8 ± 1.5	12.8 ± 1.5	–	–
neuropeptide Y	13.6 ± 0.6	13.6 ± 0.6	–	–
30 min after saline or neuropeptide Y infusion				
Control	11.0 ± 1.7	11.0 ± 1.7	–	–
neuropeptide Y	11.9 ± 0.5	11.9 ± 0.5	–	–
End of clamp				
Control	0.67 ± 0.95[b]	24.3 ± 1.6[b]	14.4 ± 0.8	9.8 ± 0.7
neuropeptide Y	0.81 ± 0.33[b]	27.8 ± 1.3[a,b]	18.9 ± 1.6[a]	9.2 ± 2.1

Mean ± SE
* See calculation section for details
Statistical analysis performed by ANOVA
[a] $p < 0.05$ compared with control group. [b] $p < 0.05$ compared with baseline value

0 min, at 1 min intervals during the first 7 min and then every 5–10 min. Blood samples for insulin determination were collected at times –30 and 0 min to assess basal insulinaemia, at times 1, 2, 3, 4, 5, 6, 7 and 10 min to assess early insulin response to hyperglycaemia and then at 10 min intervals to evaluate late insulin response. The experiments were stopped at time 120 min. During hyperglycaemic clamp, endogenous glucose production, is completely suppressed [21], and glucose disposal was determined as described in the calculations section. Early (0–10 min), late (10–90 min) and total (0–90 min) insulin responses were calculated as the area under the curve of the plasma insulin concentration during each time interval.

Effects of neuropeptide Y on systemic haemodynamics. In a separate experiment, four rats were anaesthetised with pentobarbital and prepared as previously described [22]. Mean arterial pressure and heart rate were determined by a microcomputer system (Cardiomax IIR, Columbus Instruments, Columbus, Ohio, USA) and recorded in a multichannel system (MX4P and MT4, Lectromed Ltd., Jersey, Channel Islands, UK). After baseline determination of arterial pressure and heart rates, animals received an intravenous infusion of neuropeptide Y at the same dose as in the clamp studies.

Reagents and analytical procedures. Human neuropeptide Y was purchased from Inalco (Milan, Italy), dissolved in citrate buffer and stored at –80 °C. The solution was then diluted in phosphate buffer for the experiments. 3-^{3}H-glucose and 2-deoxy [1-^{3}H]-glucose were purchased from Amersham (High Wycombe, England). Plasma glucose was determined by the glucose oxidase method (Glucose Analyzer 2, Beckman, Beckman Instruments, Palo Alto, Calif., USA). Lactate and free fatty acids were measured using commercial kits (Boehringer Mannheim, Mannheim, Germany), with enzymatic spectrophotometric techniques. Insulin was measured with a radioimmunoassay, using rat and human insulin as standards. Neuropeptide Y was measured by enzyme-immunoassay using a commercial kit (Peninsula, San Careos, Calif., USA). Plasma leptin was measured by a specific radioimmunoassay previously described in detail [23].

Calculations and statistical analysis. Glucose turnover in basal and clamp conditions was calculated as the ratio between the tracer infusion rate ($dpm \cdot min^{-1}$) and glucose specific activity ($dpm \cdot mg^{-1}$). Glucose uninary losses were measured and found to be negligible, and glucose space corrections due to changes in glucose concentration during the euglycaemic clamp were trivial. Endogenous glucose production was calcu-

lated as the difference between glucose disappearance and the exogenous glucose infusion rate. The rate of whole body glucose disposal (M) was derived from the equation M = GIR-SpC-UrL, where GIR is exogenous glucose infusion rate, SpC is the space correction during the last 20 min of clamp due to over- or underfilling of the glucose space and UrL is the glucose urinary loss during the study. Under hyperglycaemic clamp conditions the whole body glucose disposal was considered equal to the glucose infusion rate at steady state hyperglycaemia.

All data are expressed as the mean ± SE values. Statistical analysis was performed using the one way analysis of variance (ANOVA).

Results

Biochemical profiles in basal and insulin-stimulated conditions are summarised in Table 1. In the basal state, no difference was found between the control and neuropeptide Y-infused group in relation to the lactate, non-esterified fatty acid or insulin values. The rise in plasma lactate that was clearly evident during hyperinsulinaemia in control animals was suppressed in rats given neuropeptide Y (1.47 ± 0.09 vs 1.09 ± 0.06 mmol/l; $p < 0.01$). Plasma non-esterified fatty acids were similarly suppressed in both groups during hyperinsulinaemia (269 ± 39 vs 260 ± 41 μmol/l). Neuropeptide Y values were comparable in the basal state and remained essentially constant in the control experiments while being increased about fourfold in animals given neuropeptide Y infusion.

Parameters of basal and insulin-stimulated glucose metabolism are summarised in Table 2. Endogenous glucose production during basal conditions was not significantly different in the two groups of animals and was not significantly modified by a 30 min infusion of saline or neuropeptide Y (11.0 ± 1.7 vs 11.9 ± 0.5 $mg \cdot min^{-1} \cdot kg^{-1}$, NS). In addition endogenous glucose production at the end of the clamp was suppressed similarly in both groups (Table 2). As further shown by Table 2, glucose disposal at the end of clamp was higher in rats infused with neuropeptide Y than controls. Whole body glycolytic flux was increased to the same extent as total glucose disposal in the neuropeptide Y-infused group, while no significant difference was observed in glucose storage. The glucose utilisation index, measured 30 min after a bolus injection of 2DG, was not significantly different between the two groups in subcutaneous adipose tissue and in visceral adipose tissue (Fig. 1). However, in most skeletal muscles studied, there was an increase in the insulin-stimulated glucose utilisation index in the neuropeptide Y-infused group compared to controls (Fig. 1). Plasma leptin under basal conditions was 0.49 ± 0.11 and 0.63 ± 0.08 ng/ml in control and neuropeptide Y-infused rats respectively (NS). The plasma leptin concentration was increased comparably by the euglycaemic-hyperinsulinaemic clamps in

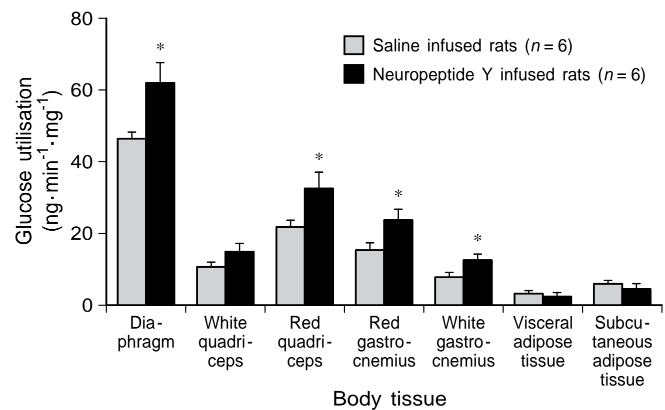

Fig. 1. Glucose utilisation index (euglycaemic-hyperinsulinaemic clamps associated with the labeled 2-deoxyglucose technique, see Methods) by muscles of control and neuropeptide Y-infused rats. Values are mean ± SE. Statistical analysis performed by ANOVA $*p < 0.05$ at least

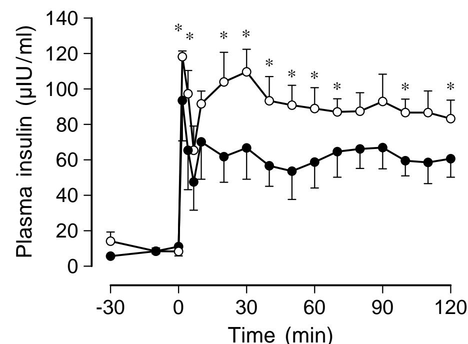

Fig. 2. Insulin output during in vivo glucose-elicited hyperglycaemic clamps in control rats (open circles, $n = 6$) and neuropeptide Y-infused (closed circles, $n = 6$) rats. Early insulin, late insulin and total insulin responses for control and neuropeptide Y-infused rats respectively were 496 ± 56 vs 321 ± 79 $\mu U \cdot ml^{-1} \cdot 10\ min^{-1}$, $p < 0.05$; 10400 ± 754 vs 6274 ± 1255 $\mu U \cdot ml^{-1} \cdot 110\ min^{-1}$, $p < 0.05$ and 10896 ± 782 vs 6595 ± 1329 $\mu U \cdot ml^{-1} \cdot 120\ min^{-1}$, $p < 0.05$. Values are mean ± SE

control and NPY-infused rats (intergroup differences, NS, differences from baseline values, $p < 0.01$ by ANOVA).

In vivo insulin output and the total rate of glucose disposal were measured during hyperglycaemic clamps. Upon doing so, plasma glucose was increased from baseline values to values of 13–13.5 mmol/l in both groups of animals. Total glucose disposal during hyperglycaemic clamps, measured in the last 30 min of the study, was unchanged by neuropeptide Y infusion, as was the glucose clearance rate (Table 3). Basal insulin concentrations and the insulin response to hyperglycaemia are depicted in Fig. 2. Neuropeptide Y treatment inhibited both the early and late insulin responses to hyperglycaemia, with the result that total insulin response was also inhibited.

Infusion of neuropeptide Y did not change the mean blood pressure (130.6 ± 3.2 vs 131.1 ±

Table 3. Biochemical profiles and parameters of glucose metabolism as assessed at the end of hyperglycaemic clamp in saline ($n = 6$) and neuropeptide Y ($n = 6$) infused animals

Group	Lactate mmol · l^{-1}	Non-esterified fatty acid (μmol · l^{-1})	Glucose disappearance rate (M)* (mg · kg^{-1} · min^{-1})	Glucose clearance (ml · kg^{-1} · min^{-1})
Control	2.34 ± 0.37	558 ± 59	19.8 ± 1.7	7.98 ± 0.64
Neuropeptide Y	2.67 ± 0.42	442 ± 62	18.1 ± 2.5	7.20 ± 1.05

Mean ± SE
* See calculation section for details

3.8 mmHg for control and treatment groups respectively) or heart rate (412 ± 15 vs 423 ± 17 bpm) from baseline values.

Discussion

In the present experiments, infusion of neuropeptide Y in overnight fasted rats lead to a fourfold increase in the arterial concentration of neuropeptide Y that remained constant throughout the clamp study. A similar increase in the plasma neuropeptide Y concentrations has been reported during muscular exercise or in stressful situations in both animals and humans [24–26].

In this study, hepatic glucose production in both basal conditions and at the end of clamp, was not influenced by intravenous neuropeptide Y infusion. A normal insulin sensitivity in suppressing hepatic glucose production was observed in both control and neuropeptide Y-infused rats. However, peripheral glucose disposal, as assessed by the clamp technique, was increased appreciably by neuropeptide Y infusion, suggesting that this peptide affected glucose uptake and utilisation by increasing their responsiveness to insulin. We cannot exclude the possibility that a longer infusion of neuropeptide Y in basal conditions would modify glucose turnover and insulin sensitivity. The predominant site of insulin-mediated whole body glucose disposal in vivo was found to be skeletal muscle. Indeed, the glucose utilisation index determined in a wide range of skeletal muscle groups comprising white, mostly red, and mixed muscle fibres was shown to be increased by neuropeptide Y infusion. It was observed, concomitantly that neuropeptide Y increased the glycolytic flux, while the rate of glucose storage remained unaffected. This observation suggests that neuropeptide Y infusion may result in direct or indirect activation not only of glucose uptake and phosphorylation but in the acceleration of some glycolytic enzyme activity at the site.

The plasma lactate concentration increased during euglycaemic hyperinsulinaemic clamps, presumably as a result of an increased glycolytic flux. During neuropeptide Y infusion, the rise in blood lactate was blunted. The present results do not allow any conclusion about the effect of neuropeptide Y on lactate metabolism, but we suggest that the neuropeptide may accelerate oxidative pathways, particularly at the level of the liver [16], thereby reducing lactate production.

In the present study, neuropeptide Y infusion failed to affect insulin-mediated glucose utilisation by white adipose tissue. This is in keeping with the lack of receptors specific to neuropeptide Y in rat adipocytes or with a low binding affinity as shown in adipocytes from dogs and humans in which neuropeptide Y displays a very weak antilypolitic effect [27, 28].

The effect of neuropeptide Y on muscular glucose metabolism may be a direct one or it may be mediated by other factors, since there is no evidence for the existence of neuropeptide Y receptors in skeletal muscle cells. On the other hand, specific Y_1 receptor subtypes have been identified in vascular cells of skeletal muscle in the pig [29], and there is increasing evidence, in both animals and humans, that neuropeptide Y may represent the non-adrenergic constrictory component that influences appreciably local blood flow in several tissues, including skeletal muscles [30, 31].

In our experiments, we did not measure regional haemodynamic responses to neuropeptide Y, but infusion did not modify either the heart rate or arterial blood pressure, suggesting that, at the doses used, neuropeptide Y had no cardiovascular effect in anaesthetised animals. The lack of effect on the systemic blood pressure and heart rate does not exclude the possibility that local neuropeptide Y effects may induce changes in regional blood flow, as previously described [32]. However, we have to consider that if the main haemodynamic effect of neuropeptide Y in the skeletal muscle vascular bed was a reduction in blood flow, the insulin and fuel supply to the cells would be reduced, resulting in reduced glucose utilisation. This is not the case in our experiments, in which neuropeptide Y has opposite effects, thus re-enforcing the conclusion of a genuine effect independent of changes in blood flow. In any case, we cannot exclude the possibility that the effect on glucose metabolism is mediated centrally since leakage through the blood brain barrier has been reported [33].

Our study also shows the effect of neuropeptide Y on insulin secretion kinetics as assessed during hyperglycaemic clamps. Intravenous neuropeptide Y inhibited both the early and the late phase of insulin response. It is noteworthy that this effect is evident at concentrations appreciably lower than those reported

to inhibit insulin release in in vitro studies [14, 34, 35]. In mouse pancreas neuropeptide Y mRNA is present in the islets of Langerhans at the same site as glucagon within the alpha-cells [36, 37]. Moreover a nervous network rich in neuropeptide Y as well as sympathetic nerve endings containing the peptide have been found in the endocrine portion of the pancreas [38], suggesting that neuropeptide Y has a role in the neural regulation of islet function as a mediator of sympathetic nervous system induced inhibition of insulin secretion.

Our results are consistent with findings from previous in vitro studies indicating that neuropeptide Y has a prevalent inhibitory effect on insulin secretion [14, 34, 35]. This suggests that neuropeptide Y may inhibit insulin secretion by a direct pancreatic action. However, in vivo studies of the affects of neuropeptide Y on insulin output have often given contradictory results. In rats, intravenous neuropeptide Y stimulated basal insulin secretion in vivo, while inhibiting glucose-induced insulin secretion [39]. Moreover, in dogs, the pancreatic administration of neuropeptide Y resulted in a slight stimulatory effect [40].

Given the vasocontrictory effect of neuropeptide Y, regional reduction in blood flow could justify a reduced pancreatic hormone production in keeping with other data that showed a reduction in pancreatic flow and insulin secretion with neuropeptide Y in pigs [41] and in perfused rat pancreas [42]. In our protocol, we observed no change in arterial blood pressure and heart rate, suggesting that another mechanism must underly the effect of neuropeptide Y on insulin output. It is well known that norepinephrine is a powerful inhibitor of insulin secretion and neuropeptide Y influences the sympathetic tone [43]. Thus, neuropeptide Y may exert its inhibitory role on insulin secretion indirectly through its action on norepinephrine, rather than directly.

We have previously observed that long term intracerebroventricular neuropeptide Y administration in rats induces a syndrome characterised by increased food intake, obesity, hyperinsulinaemia, muscle insulin resistance and an increased metabolic activity in white adipose tissue [1–3]. It has been widely recognised that *ob* gene expression and leptin release by adipose tissue are under neuroendocrine influence, and it has been shown that intracerebroventricular neuropeptide Y infusion also increases *ob* gene expression [6] and its product, which may in turn inhibit neuropeptide Y at the central nervous system level [7]. Furthermore, leptin expression and production by adipose tissue are regulated by factors influencing glucose metabolism, such as insulin and glucocorticoids. In particular, insulin rapidly regulates *ob* gene transcription, enhancing circulating leptin in rats [23, 44, 45]. In our experiments, we observed that continuous intravenous infusion of insulin during euglycaemic clamp produced a significant increase in serum leptin, as reported previously [23]. Moreover the intravenous infusion of neuropeptide Y did not induce any change in leptin concentrations, either in basal conditions or during euglycaemic hyperinsulinaemia. In addition, intracerebroventricular neuropeptide Y has been shown to potentiate the insulinaemic response to intravenous glucose, while reducing the ability of insulin to control glucose metabolism and, in particular, to suppress glucose production [46, 47].

In conclusion, the major new finding of this study is the observation that peripheral neuropeptide Y infusion in normal rats increases the overall rate of glucose disposal by increasing insulin responsiveness in skeletal muscle. Moreover, the increased glycolytic flux combined with the blunted increase in lactate suggests that neuropeptide Y may raise insulin mediated glucose disposal by increasing its utilisation through the oxidative pathways. Further in vitro studies are required to confirm this hypothesis. Intravenous neuropeptide Y does not influence glucose metabolism in adipose tissue and leptin release. Peripheral neuropeptide Y plays a clear inhibitory role in glucose-induced insulin secretion. These data indicate that neuropeptide Y has different effects on insulin secretion when administered acutely via intracerebroventricular or intravenous routes. In addition, the effects on peripheral and hepatic glucose metabolism are different between these routes, but it is possible that the duration of treatment, and not just the route of administration, may be a relevant factor.

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