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Anorectic effect of metformin in obese Zucker rats: lack of evidence for the involvement of neuropeptide Y

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Abstract

The hypothalamic neuropeptide Y content and preproneuropeptide Y mRNA expression were studied in metformin-treated (300 mg/kg orally for 12 days), in pair-fed and in ad libitum-fed obese Zucker rats in order to elucidate possible mechanisms involved in the anorectic and body weight reducing effect of chronic metformin treatment in genetically obese Zucker rats. In addition the acute influence of metformin on food intake was studied by comparing its effects after oral and parenteral administration. The concentration of neuropeptide Y in the hypothalamic paraventricular nucleus was significantly higher in the metformin-treated and pair-fed rats when compared to the control animals. The expression of preproneuropeptide Y mRNA in the arcuate nucleus was similar in all three treatment groups. Both chronic metformin treatment and pair-feeding markedly lowered hyperinsulinaemia in these animals. A single subcutaneous dose of metformin (300 mg/kg) reduced food intake only in obese animals, while the same dose of metformin given orally did not affect food intake in either lean or obese animals. It is concluded that the treatment with metformin and pair-feeding, which results in comparable reductions in food intake, body weight gain and hyperinsulinaemia, similarly increase neuropeptide Y concentrations in the paraventricular nucleus while not affecting preproneuropeptide Y mRNA expression in the arcuate nucleus. The increase in hypothalamic neuropeptide Y content may be secondary to the reduction in hyperinsulinaemia during metformin treatment and pair-feeding. Thus, the anorectic effect of chronic metformin treatment cannot be explained by changes in content or expression of hypothalamic neuropeptide Y.

Keywords: Metformin; Zucker rat; Genetic obesity; Feeding behavior; Insulin; Neuropeptide Y

1. Introduction

The biguanide metformin is an antihyperglycaemic agent widely used to treat obese type 2 diabetic patients particularly because of its weight reducing and serum lipid profile normalizing effects (Hermann, 1979; Vigneri and Goldfine, 1987; Bailey, 1992). The therapeutic effects of metformin have been attributed to an improved peripheral glucose utilization and insulin sensitivity (Klip and Leiter, 1990; Bailey, 1992), but its cellular mode of action is still incompletely understood. In skeletal muscle metformin seems to potentiate the effects of insulin on glucose uptake (Klip and Leiter, 1990) and in vitro it causes translocation of glucose transporter proteins from the intracellular compartment to the plasma membrane (Klip and Leiter, 1990; Hundal et al., 1992). The mechanisms by which metformin treatment lowers body weight are still unknown (Bailey, 1992). We have recently reported that chronic metformin treatment reduces food intake but does not affect brown adipose tissue thermogenetic activity in genetically obese Zucker rats (Rouru et al., 1992, 1993b). Therefore the weight gain reducing effect of metformin seems to be determined at least partly by

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reduced caloric intake. However, the mechanism for this food intake reducing effect remains to be clarified.

Food intake is regulated by complex mechanisms in which various hypothalamic neurotransmitters and particularly neuropeptide Y play a prominent role (Morley, 1987; Blundell, 1991; Leibowitz, 1991). The hypothalamic paraventricular nucleus is heavily innervated by neuropeptide Y-containing neurons which originate from the arcuate nucleus, medulla and dorsal pons (Bai et al., 1985; Leibowitz, 1991). Acute and chronic injections of neuropeptide Y into the paraventricular nucleus elicit a long-lasting hyperphagic effect and neuropeptide Y has been regarded as the most potent naturally occurring orexigenic agent (Leibowitz, 1991; Sahu and Kalra, 1993). A feature which has thus far distinguished neuropeptide Y from other candidate regulators of food intake is that its production within the arcuate nucleus, and its release into the paraventricular nucleus, is affected by the energy balance of the animal (Schwartz et al., 1992). Food-deprived and streptozotosin-diabetic rats, with low peripheral insulin, have increased hypothalamic neuropeptide Y and preproneuropeptide Y mRNA levels (Williams et al., 1989; Brady et al., 1990; Schwartz et al., 1992; Sahu and Kalra, 1993). Furthermore, neuropeptide Y antisense oligodeoxynucleotides injected into the arcuate nucleus suppress food intake (Akabayashi et al., 1994), which suggests a physiological role for neuropeptide Y in the control of feeding behaviour (Schwartz et al., 1992; Sahu and Kalra, 1993). Intracerebroventricularly administered insulin prevents the fasting induced increase in hypothalamic levels of both neuropeptide Y peptide and its mRNA, and centrally administered insulin decreases food intake (Schwartz et al., 1992). Thus, insulin seems to be one important regulator of hypothalamic neuropeptide Y secretion and therefore neuropeptide Y is supposed to be the link connecting insulin to the regulation of food intake (Schwartz et al., 1992; Sahu and Kalra, 1993).

The purpose of the present study was to further characterize the anorectic action of metformin in genetically obese Zucker rats by comparing its effects after single parenteral and oral doses and by investigating how chronic metformin treatment affects hypothalamic neuropeptide Y content and its mRNA expression. The involvement of the hypothalamic neuropeptide Y system was considered as a potential candidate for the anorectic effect of metformin in the light of the interrelationship between plasma insulin and neuropeptide Y and the reduction of hyperinsulinaemia in obese Zucker rats during metformin treatment. Because these animals are hyperinsulinaemic, hyperphagic, hyperlipaemic and markedly insulin resistant (Bray et al., 1989), they resemble human obesity and type 2 diabetes mellitus, and provide a convenient animal model to study the effects of metformin.

2. Materials and methods

2.1. Animals

For the acute experiments 20 obese (*fa/fa*) and 20 lean (*Fa/−*) male Zucker rats and for the neuropeptide Y content and gene expression experiments 60 obese (*fa/fa*) male Zucker rats were purchased from Iffa Crédo (L'Arbresle, France). All rats were individually housed, maintained on a regular light-dark cycle, lights on from 6.00 a.m. to 8.00 p.m. and provided with normal laboratory rat chow (SDS rat and mouse No. 1 maintenance diet, Witham, UK) containing 10.9 MJ/kg of metabolizable energy.

2.2. Food intake after single parenteral metformin dose

The rats were 6–7 weeks old at the beginning of the experiment. All rats were acclimated for one week to diet and diurnal cycle and were habituated for 3 days to handling and subcutaneous injections before the experiment was started. Both lean and obese animals were divided to three experimental groups matched for body weight ($n = 6$ in each group). The rats were fasted for 21 h, during which they were allowed tap water ad libitum. They received a subcutaneous injection of either 0.9% saline (1 ml/kg) or metformin hydrochloride (150 or 300 mg/kg, Leiras Oy, Turku, Finland) dissolved in an equal volume of saline. Food was provided to them 25 min after the injections and the food consumed was measured after 1, 2 and 4 h.

2.3. Kinetics of metformin after parenteral dose

The rats were allowed to recover for 10 days after the previous experiment described above with food and water ad libitum. Twelve lean and 12 obese animals were divided into three groups (one group for each time point, see below) matched for body weight. They were fasted for 21 h as in the first experiment. All rats received a subcutaneous injection of metformin hydrochloride 300 mg/kg. Blood was collected into EDTA-containing tubes from four rats of each phenotype by wrapping the rats in a towel and quickly cutting the tail tip after 30, 60 or 120 min. Blood was collected from each animal only once. Plasma metformin concentration was determined by high-performance liquid chromatography as described earlier (Huupponen et al., 1992).

2.4. Food intake after single oral metformin dose

The rats were allowed to recover for 11 days after the previous experiment with food and water ad libitum. They were habituated for 3 days to handling and intragastric injections before the experiment was

started. Both lean and obese animals were divided into two experimental groups matched for body weight ($n = 6$ in each group). The rats were fasted for 21 h, during which they were allowed tap water ad libitum. They received an intragastric injection of 0.9% saline (1 ml/kg) or metformin hydrochloride 300 mg/kg dissolved in an equal volume of saline. 30 min after the injections food was provided and the food consumed was measured after 1, 2 and 4 h.

2.5. Chronic metformin treatment with pair-feeding

30 obese Zucker rats were used for the determinations of neuropeptide Y content in the arcuate nucleus and paraventricular nucleus. Another set of 30 obese rats was used for the determination of preproneuropeptide Y mRNA in the arcuate nucleus. None of these rats had received metformin before. These two animal groups were both further divided into three experimental groups (metformin-treated, pair-fed and control) matched for body weight and 24 h food intake recordings ($n = 10$ in each group). The characteristics of these groups are represented in Tables 1 and 2. The metformin-treated group received 300 mg/kg/day metformin hydrochloride dissolved in the drinking water. In our earlier study this dose was shown to be well tolerated by obese Zucker rats and devoid of any effect on their spontaneous locomotor activity (Rouru et al., 1993a). The 24-h fluid intake was monitored and the concentration of metformin in the drinking water was adjusted every second day to maintain the correct daily dose. The control group and the pair-fed group received drinking water without metformin ad libitum. Because the rats in the metformin group ate less than those in the control group, the pair-fed group received a restricted amount of food (at 16.00 h) to ensure a similar caloric intake as that of the metformin-treated rats. Food intake and body weights were measured every day. After 12 days' treatment, food and water were withdrawn from all animals at 7.00 h and they were decapitated between 9.30 h and 13.30 h. Blood was collected into prechilled EDTA-containing tubes, whereafter plasma was separated and stored at −70°C until analysed for insulin and glucose. Brains were removed quickly, frozen in isopenthane on dry ice and stored at −70°C.

2.6. Chronic metformin treatment with pair-drinking

In order to elucidate the role of reduced fluid intake on the anorectic effect of metformin treatment we divided 21 obese Zucker rats into three experimental groups (metformin-treated, pair-drinking and control) matched for body weight ($n = 7$ in each group). The metformin-treated group received 300 mg/kg/day metformin hydrochloride dissolved in the drinking water as in the experiments described above. The control group received drinking water without metformin ad libitum. Because the rats in the metformin group drank less than those in the control group, the pair-drinking group received a restricted amount of water (at 14.00 h) to ensure a similar fluid intake to that of the metformin-treated rats. Food and fluid intake was measured every day.

2.7. Measurement of hypothalamic neuropeptide Y content

Brains were cut into coronal sections of 300 μm at −12°C, and two hypothalamic areas, namely the paraventricular nucleus and arcuate nucleus, were micropunched following the coordinates given in the stereotaxic atlas of König and Klippel (1974). Bilateral tissue samples were placed in 100 μl of cold aprotinin (Iniprol, Laboratories Choay, Paris, France) in microfuge tubes and were stored at −70°C until assayed. The tissue samples were homogenised in cold 0.2 N HCl. An aliquot was taken for protein analysis and the remainder of the homogenate was centrifuged at 2500 × g for 30 min at 4°C, and the supernatant (50 μl) was then lyophilized and taken for determination of neuropeptide Y concentration.

Neuropeptide Y was measured with a specific radioimmunoassay kit using the double antibody method (Peninsula Laboratories, USA). Standard (porcine neuropeptide Y) or lyophilized extract reconstituted with 0.04 M phosphate buffer (pH 7.4) was incubated with specific neuropeptide Y antiserum and ^{125}I-labelled neuropeptide Y for 48 h including a 24 h preincubation period without labelled neuropeptide Y. Bound and free fractions were separated by the addition of a goat anti-rabbit IgG serum. The samples were measured in duplicate and the values are presented as pg/mg protein. The protein determination was performed by the method of Bradford (1976).

2.8. Measurement of hypothalamic neuropeptide Y mRNA expression

Brains were cut in a cryostat (at −18°C) into coronal 14 μm sections at the level of the arcuate nucleus (A4890). The preproneuropeptide Y mRNA in situ hybridization procedure was performed as described earlier (Pesonen et al., 1992). Brain sections were placed against Kodak X-OMAT AR film and exposed for 7–10 days at −70°C with intensifying screens and ^{14}C standards (American Radiochemicals, St. Louis, MO, USA). The autoradiography films were analysed with a OS/2 based image analysis system (MCID, Imaging Research, Ontario, Canada). For linear quantification ln(grey value of standard) was plotted against ln(radioactivity of ^{14}C standard). The grey values of

each animal were converted to radioactivity per tissue equivalent, using the standard curve, and expressed as relative radioactivity units.

2.9. Plasma insulin and glucose

Plasma insulin was measured with a rat insulin radioimmunoassay kit supplied by Novo BioLabs (Bagsvaerd, Denmark). The interassay coefficient of variation was 7.5% at 1 ng/ml and 4.6% at 2 ng/ml. Plasma glucose was analysed with the glucose oxidase method (Analox Instruments, London, UK).

2.10. Statistical analysis

Statistical analysis of the data from the acute experiments was carried out by two-way analysis of variance (ANOVA) for repeated measurements or by two-way ANOVA. When significant phenotype × treatment × time interaction effects were found, phenotypes were tested separately by ANOVA for repeated measurements. Significant treatment × time interaction effects were then assessed by one-way ANOVA separately for each time point and Bonferroni corrected contrasts were used to detect differences between the control group and metformin-treated groups. For the chronic treatment data originating from obese phenotype only, one-way ANOVA for repeated measurements or one-way ANOVA followed by the Newman-Keuls procedure for multiple comparisons was used. If necessary, logarithmic transformation of the data was performed prior to analysis. The calculations were performed using BMDP software (BMDP Statistical Software, Los Angeles, CA, USA). A P-value less than 0.05 was considered statistically significant.

3. Results

3.1. Food intake and metformin kinetics after a single metformin dose

Metformin at the dose of 300 mg/kg subcutaneously significantly reduced food intake in obese Zucker rats at 1 h ($P < 0.001$) and 2 h ($P < 0.01$), whereas 150 mg/kg had no effect (Fig. 1). After 2 h there was a clear compensatory increase in food intake in obese animals which had received the higher dose ($P < 0.05$, Fig. 1). In lean Zucker rats subcutaneous metformin did not significantly affect food intake (Fig. 1). A single intragastric metformin dose (300 mg/kg) did not acutely alter food intake in either obese or lean Zucker rats (Fig. 1).

Plasma metformin concentrations were significantly higher in obese rats than in lean rats 60 min after subcutaneous metformin injection (Table 1).

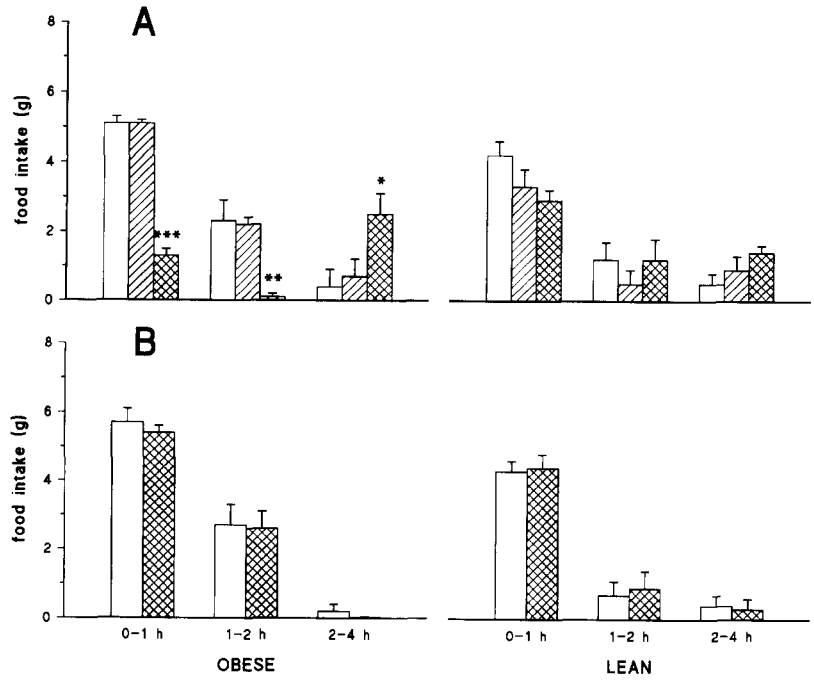

Fig. 1. Food intake after parenteral (A) and intragastric (B) administration of saline (open bars), metformin 150 mg/kg (hatched bars) and metformin 300 mg/kg (cross-hatched bars). The values are the means ± S.E.M., $n = 6$ in each group. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ significance of the difference when compared with the control group.

Table 1
Concentrations of metformin (μg/ml) in plasma after a subcutaneous metformin dose of 300 mg/kg

	30 min	60 min	120 min
Lean	218±17	137±15	32±2
Obese	225±29	225± 7 [a]	69±9

Values are means±S.E.M., $n = 4$ in each group.
[a] $P < 0.01$ significance of the difference when compared with the lean group at the respective time points; two-way ANOVA followed by Bonferroni corrected contrasts.

Table 2
Feeding behaviour parameters and plasma insulin and glucose levels in metformin-treated, pair-fed and control rats, used for the analysis of neuropeptide Y content in the arcuate nucleus and paraventricular nucleus

	Control	Metformin	Pair-fed
Initial body weight (g)	360 ± 6	361 ± 6	359 ± 5
Weight gain (g)	76 ± 3	46 ± 3 [a]	51 ± 4 [a]
Cumulative food intake (g)	416 ± 8	349 ± 8 [a]	357 ± 8 [a]
Cumulative water intake (ml)	369 ±14	258 ±10 [a,b]	323 ±11 [a]
Plasma insulin (ng/ml)	22.5± 3.1	10.0± 0.8 [a]	8.1± 1.0 [a]
Plasma glucose (mmol/l)	7.5± 0.1	7.1± 0.1 [a,b]	6.6± 0.1 [a]

Values are means±S.E.M., $n = 10$ in each group.
[a] $P < 0.05$ significance of the difference when compared with the control group, [b] $P < 0.05$ significance of the difference when compared with the pair-fed group.

3.2. Food intake, water intake and body weights during chronic metformin therapy

During the 12-day treatment the rats in the metformin and pair-fed groups ate significantly less than the rats in the control group (Tables 2 and 3). Metformin reduced food intake already after one day of treatment and the effect was still significant at the end of the experiment. The time course of the metformin effect on food intake in rats in which preproneuropeptide Y expression was measured is shown in Fig. 2. A similar effect was seen in the rats used for the determination of hypothalamic neuropeptide Y content (data not shown). Body weight gains were significantly reduced in metformin-treated and pair-fed groups when compared with the control group (Tables 2 and 3). Water intake was significantly reduced in metformin-treated rats when compared with both control and pair-fed groups (Table 2). In addition the rats of the pair-fed group consumed less water than the rats in the control group (Table 2).

In the pair-drinking experiment metformin treatment significantly reduced cumulative food and water

Table 3
Feeding behaviour parameters and plasma insulin and glucose levels in metformin-treated, pair-fed and control rats, used for the analysis of preproneuropeptide Y mRNA expression in the arcuate nucleus

	Control	Metformin	Pair-fed
Initial body weight (g)	420 ± 7	421 ± 7	419 ±11
Weight gain (g)	35 ± 3	14 ± 5 [a]	17 ± 5 [a]
Cumulative food intake (g)	335 ±10	284 ±15 [a]	292 ±13 [a]
Plasma insulin (ng/ml)	17.1± 2.0	10.3± 1.4 [a]	10.2± 1.0 [a]
Plasma glucose (mmol/l)	7.8± 0.1	8.3± 0.3	7.9± 0.2

Values are means±S.E.M., $n = 10$ in each group.
[a] $P < 0.05$ significance of the difference when compared with the control group.

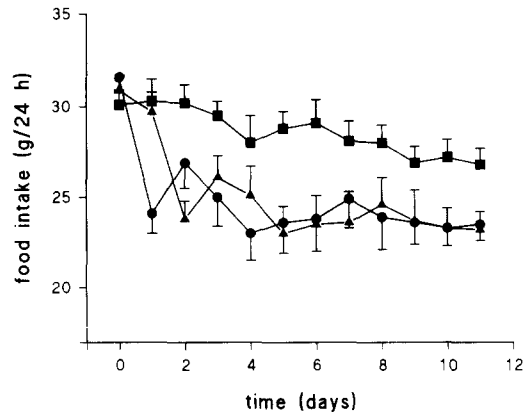

Fig. 2. 24 h food intake in control (squares), metformin-treated (circles) and pair-fed animals (triangles). The values are the means± S.E.M., $n = 10$ in each group. After first treatment day: control vs. metformin group $P < 0.05$. After eleventh treatment day: control vs. metformin group $P < 0.05$, control vs. pair-fed group $P < 0.05$. Other pairwise comparisons were non-significant (ANOVA followed by Newman-Keuls procedure).

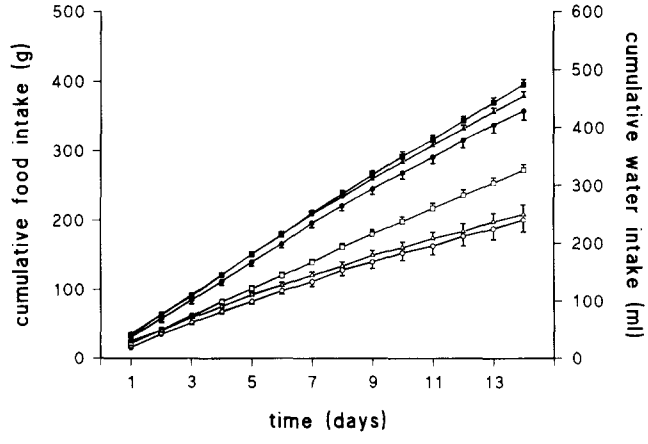

Fig. 3. Time course of cumulative food (solid symbols) and fluid intake (open symbols) in control (squares), metformin-treated (circles) and pair-drinking animals (triangles). The values are the means± S.E.M., $n = 7$ in each group. Cumulative food intake after 14 treatment days: control vs. metformin group $P < 0.05$. Cumulative fluid intake after 14 treatment days: control vs. metformin group $P < 0.05$, control vs. pair-drinking group $P < 0.05$. Other pairwise comparisons were non-significant (ANOVA followed by Newman-Keuls procedure).

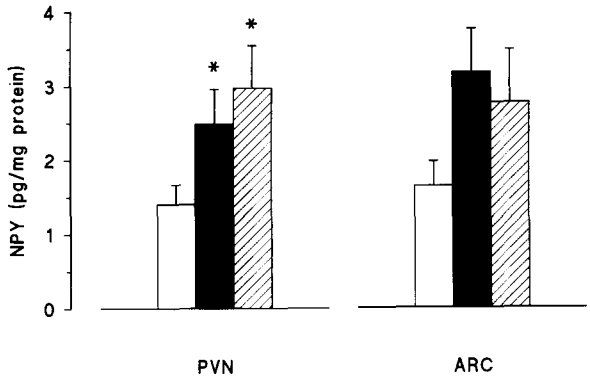

Fig. 4. Neuropeptide Y contents of the paraventricular and arcuate nuclei in control (open bars), metformin-treated (solid bars) and pair-fed animals (hatched bars). The values are the means ± S.E.M., $n = 5–10$ in each group. * $P < 0.05$ significance of the difference when compared with the control group.

intake when compared to that of the control group (Fig. 3). In addition, body weight gain was significantly ($P < 0.05$) reduced in the metformin group only (control group: 61 ± 2 g, metformin group: 28 ± 6 g, pair-drinking group 49 ± 14 g, mean ± S.E.M.). Cumulative food intake and body weight gain did not differ significantly between control and pair-drinking groups even after 14 days' treatment.

3.3. Neuropeptide Y content and expression

Neuropeptide Y content was higher in the paraventricular nucleus of metformin-treated ($P < 0.05$) and pair-fed rats ($P < 0.05$) than in control animals (Fig. 4). In the arcuate nucleus of metformin-treated and pair-fed rats, the neuropeptide Y content was also higher than in control animals, but these differences did not quite reach statistical significance ($P = 0.067$, ANOVA, Fig. 4). There was no significant difference in the expression of preproneuropeptide Y mRNA in the arcuate nucleus between metformin-treated, pair-fed and control animals (Fig. 5).

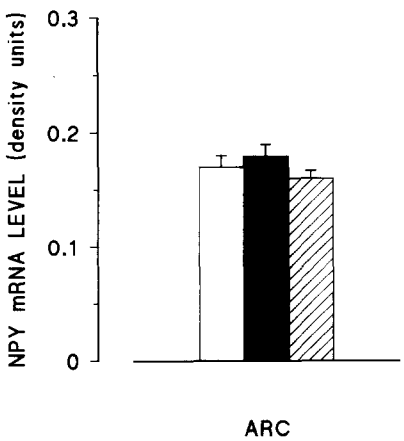

Fig. 5. Preproneuropeptide Y mRNA levels in the arcuate nucleus in control (open bars), metformin-treated (solid bars) and pair-fed animals (hatched bars). The values are relative density units (means ± S.E.M., $n = 9–10$ in each group).

3.4. Plasma insulin and glucose

Fasting plasma insulin concentrations were significantly lower in both chronically metformin-treated and pair-fed rats when compared with the control group (Tables 2 and 3). Plasma glucose levels were slightly but significantly lower both in metformin-treated and in pair-fed groups than in the control group in the animals used for analysis of hypothalamic neuropeptide Y content (Table 2). No difference existed in the slightly heavier Zucker rats used for the preproneuropeptide Y mRNA analysis (Table 3).

4. Discussion

The main finding of the present study is that the content of neuropeptide Y is increased in the paraventricular nucleus and arcuate nucleus of both metformin-treated and pair-fed obese Zucker rats while neuropeptide Y mRNA expression is not altered in the arcuate nucleus. The changes in neuropeptide Y content in the pair-fed animals are consistent with the mild food restriction and with the known feeding stimulatory role of neuropeptide Y under such dietary situations. Therefore, similar effects in the metformin-treated animals, which have a reduced food intake, suggest that other, possibly peripherally driven mechanisms which are capable of counteracting neuropeptide Y generated systems contribute to the anorectic effect of metformin.

The genetically obese Zucker rat is hyperphagic, hyperinsulinaemic and markedly insulin resistant (Bray et al., 1989). The reason for the development of the obesity syndrome in these animals is unknown, but it has been suggested to be of central origin (Bray et al., 1989). Obese rats have an abnormally low insulin level (Figlewicz et al., 1985) and insulin receptor binding in the brain (Figlewicz et al., 1985), but increased insulin content in the cerebrospinal fluid (Figlewicz et al., 1985). The neuropeptide Y content in the paraventricular nucleus and neuropeptide Y mRNA levels in the arcuate nucleus are higher in obese than in lean animals (McKibbin et al., 1991; Pesonen et al., 1992). The obese rats have also a decreased glucose utilization in the whole brain and in various specific brain areas, including the lateral hypothalamus, when compared with lean Zucker rats (Marfaing-Jallat et al., 1991). Although glucose utilization in the central nervous system is not principally dependent on insulin, the above finding suggests that an insulin resistant state in

the periphery can be reflected also in the central nervous system. In agreement with this, obese Zucker rats have a reduced sensitivity to the food intake- and body weight-reducing effect of centrally administered insulin (Ikeda et al., 1986).

In this study and in previous studies (Rouru et al., 1992, 1993a,b) metformin treatment has proven to have an anorectic effect that leads to reduced body weight gain in obese Zucker rats. Because metformin potentiates the action of insulin (or acts in a way resembling it) in the periphery (Bailey, 1992) and leads to lowered hyperinsulinaemia during chronic treatment, it was hypothesized that metformin could also potentiate the feedback effect of insulin on neuropeptide Y synthesis in the arcuate nucleus and neuropeptide Y concentration in the paraventricular nucleus. Such a potentiation could be one explanation for metformin's anorectic effect. However, in this study metformin did increase the content of neuropeptide Y in both the paraventricular nucleus and arcuate nucleus, which is contrary to our initial hypothesis. The observation that the content of neuropeptide Y was increased in both the paraventricular nucleus and arcuate nucleus in the pair-fed group as well suggests that this increment was due to decreased insulin concentrations in plasma both in the metformin-treated and pair-fed groups.

Pair-feeding can be interpreted as mild food restriction. According to previous studies, food restriction increases preproneuropeptide Y mRNA expression in the arcuate nucleus (Brady et al., 1990), an effect which is similar in obese and lean Zucker rats (Pesonen et al., 1992), and food deprivation increases the neuropeptide Y content in the paraventricular nucleus and preproneuropeptide Y mRNA expression in the arcuate nucleus (Brady et al., 1990; Jhanwar-Uniyal and Chua, 1993; Sahu and Kalra, 1993). In the present study we failed to find any difference in the expression of neuropeptide Y mRNA between treatment groups. The dissociation between neuropeptide Y concentrations and its mRNA expression suggests that the increment in neuropeptide Y peptide concentration is not due to increased synthesis of neuropeptide Y at a transcriptional level but rather reflects posttranscriptional regulation of neuropeptide Y synthesis. It should be also noted that the paraventricular nucleus receives neuropeptide Y innervation from the medulla and pons, where we did not investigate preproneuropeptide Y mRNA expression. Finally, the in situ hybridization technique is only semiquantitative and it is possible that it is not sensitive enough to find effects of mild food restriction on neuropeptide Y synthesis.

Water intake was significantly reduced in the metformin-treated rats when compared with both the control and pair-fed rats. In addition, the rats in the pair-fed group consumed less water than the control rats. However, the weight gain in the metformin-treated and pair-fed rats was similar despite the smaller fluid intake in the former (Table 2), indicating that the weight gain was correlated predominantly with food intake. When a group of rats were given a similar amount of water as that consumed by the rats in the metformin group, cumulative food intake or body weight gain did not differ significantly between the control and the pair-drinking groups, which agrees with the view that metformin's effect on food intake and body weight is not mediated to any significant degree by fluid intake. As demonstrated in Figs. 2 and 3, the anorectic effect of metformin began within 1–3 days after the start of the treatment while food intake was only slightly affected by the end of 14 days' treatment in the pair-drinking group. Although the control of food and water intake is linked, for example by neuropeptide Y, which increases food and water intake after injection into hypothalamus (Grundemar and Håkanson, 1994), the above results strongly suggest that metformin's anorectic action is a primary effect and not secondary to the reduced fluid intake.

Chronic metformin treatment has been shown to reduce food intake and body weight gain in both lean and obese Zucker rats, but this effect is more clearly seen in obese animals (Rouru et al., 1992). A single intraperitoneal dose of metformin 250 mg/kg is anorectic in genetically obese (*ob/ob*) and lean (*ob/−*) mice but this effect is more rapid and more protracted in obese animals (Bailey et al., 1986). This is in line with the present study where acute subcutaneous metformin administration (300 mg/kg) transiently reduced food intake, the effect being more pronounced and statistically significant in obese rats only. However, single intragastric metformin administration of the same dose did not have any effect on food intake, contrasting with the findings after chronic administration.

The concentrations of metformin in plasma after subcutaneous administration were very high when compared with the concentrations after normal maintenance therapy in humans (Charles et al., 1981) or during oral 2-week therapy with 320 mg/kg/day metformin in genetically obese Zucker rats (Rouru et al., 1993a). The difference between the phenotypes after single subcutaneous doses could be at least partly explained by differences in the pharmacokinetics of metformin between lean and obese rats. Based on an analysis of plasma metformin concentrations, it seems probable that the anorectic effect of acute subcutaneous metformin in the present study involves unspecific, possibly even toxic mechanisms which are different to those active during chronic oral therapy. It should be noted that there were no signs of a toxic effect during chronic metformin treatment. Furthermore, the lack of an acute effect on feeding after the large single oral dose suggests that rats tolerate oral

metformin without significant gastrointestinal symptoms affecting feeding.

It is concluded that metformin therapy and pair-feeding similarly increase neuropeptide Y content in the paraventricular nucleus and arcuate nucleus while not affecting preproneuropeptide Y mRNA expression in the arcuate nucleus in genetically obese Zucker rats. The increase in the hypothalamic neuropeptide Y content may be associated with the reduction in hyperinsulinaemia observed during metformin treatment and pair-feeding. Thus, the anorectic effect of metformin cannot be explained by changes in the content or expression of hypothalamic neuropeptide Y.

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