Indices of Carbohydrate and Lipid Metabolism in Vasopressin-Replete and -Deficient New Zealand Genetically Hypertensive Rats

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Indices of carbohydrate and lipid metabolism were investigated in male New Zealand genetically hypertensive and normotensive rats. Cross-breeding of male rats of these strains with female Brattleboro diabetes insipidus rats also provided the opportunity to examine the metabolic impact of vasopressin and its deficiency in hypertensive and normotensive rats. Hypertensive and normotensive rats, with or without diabetes insipidus, were fasted for 24 h, exsanguinated and their blood/plasma analysed for various indices of carbohydrate and lipid metabolism. Whilst each group of rats maintained fasted normoglycemia, hypertensive rats, with or without vasopressin-deficiency, were hypoinsulinaemic relative to normotensive counterparts. Moreover, hypertensive or normotensive vasopressin-deficient rats were hypoinsulinaemic relative to vasopressin-replete counterparts. In vasopressin-replete rats, the apparently improved insulin sensitivity in hypertension was associated with significant falls in plasma glucagon, triglycerides and total cholesterol. Finally, normotensive vasopressin-deficient rats were hypoglucagonaemic relative to the vasopressin-replete group. These data demonstrate that independent of vasopressin status, hypertension in the New Zealand strain and the diabetes insipidus substrains, the AVP-deficient genetically hypertensive (DI/H) and normotensive (DI/N) rat.

Materials and Methods

Animals

Male NZGH, NZN, DI/H and DI/N rats aged 12–14 weeks (280–360 g) were bred in the Animal Unit, University of Manchester (U.K.) and their blood pressure measured by tail cuff plethysmography as described previously [11]. Animals were maintained on a 12 h light/12 h dark regimen and allowed free access to standard pelleted rat chow (SDS, Witham, Essex, UK) and tap water.

Experimental protocol

Animals were fasted from 10 a.m. and killed by decapitation in the interval 9.30–11.30 a.m. on the following day. Trunk blood was collected and chilled and the blood glucose concentration measured immediately (2300 Stat Yellow Springs Glucose Analyzer). The remaining blood was centrifuged and separated plasma was stored at –20 °C, awaiting further analysis. Standard commercial kits were used for determination of insulin (Amersham International, Amersham U.K.), glucagon (Eur/DCF, Caernarfon, Gwynedd, U.K.), triglycerides and total cholesterol (Boehringer Mannheim, Germany). Plasma corticosterone was measured by radioimmunoassay of ethanol-extracted samples [12]: inter- and intra-assay coefficients of variation were 10.1% (n = 30) and 13.6% (n = 10), respectively.

Key words: Rat - Vasopressin - Insulin - Metabolism - Hypertension

Introduction

The neurohypophysial hormones vasopressin (AVP) and oxytocin (OT) and vasopressin receptors are present in rat and human pancreas [1,2,3,4]. Moreover, in both in vivo and in vitro studies, AVP and OT have been shown to stimulate insulin and glucagon secretion [5,6,7,8,9], suggesting the possibility of a role for these hormones in glucose metabolism.

In the obese Zucker rat, a genetic model of obesity and insulin resistance, the plasma AVP concentration is elevated when compared with its lean counterpart [10], and this raises the question of whether dysregulation of AVP secretion may play a role in the development and maintenance of the cluster of metabolic abnormalities seen in this animal model. To investigate the relationship between AVP and glucose and lipid metabolism further, we studied animal models in which plasma AVP is abnormally elevated or decreased, namely the AVP-replete New Zealand genetically hypertensive (NZGH) rat, its normotensive counterpart (NZN) and their cross-bred diabetes insipidus substrains, the AVP-deficient genetically hypertensive (DI/H) and normotensive (DI/N) rat [11].
Statistical analysis

All values have been expressed as means ± SEM. Differences between hypertensive and normotensive rats and between AVP-replete and AVP-deficient animals were established using a one-way analysis of variance followed by the Student-Newman-Keuls multiple-comparison test. A probability value (p) of less than 0.05 was considered statistically significant.

Results

Figures 1 and 2 summarize values for basal and fasted plasma insulin, glucagon and blood glucose concentrations, and plasma triglycerides and total cholesterol concentrations in each group of rats. Values for the 24 h fasted blood glucose levels were similar in each of the 4 groups (see Figs. 1 and 2). Moreover, compared with the AVP-replete substrains, AVP-deficiency was associated with significantly lower fasted plasma insulin concentrations in both hypertensive and normotensive animals. The insulin/glucose ratio was also significantly lower in NZGH (261.8 ± 27.5 ng/mmol) than in NZN (380 ± 41.1 ng/mmol) rats. In rats with diabetes insipidus, the insulin/glucose ratio was slightly, but not significantly, lower in DI/H (170 ± 12.3 ng/mmol) than in DI/N (221 ± 35.2 ng/mmol).

Fig. 1 Blood glucose, plasma total cholesterol and triglyceride (presented in mmol/l) and plasma insulin and glucagon (presented in ng/ml and pg/ml respectively) concentrations in 24 h fasted AVP-replete New Zealand genetically normotensive (NZN) and hypertensive (NZGH) rats. Values are mean ± SEM. n = animal number. Statistical comparisons between NZN and NZGH are by one-way analysis of variance followed by Student-Newman-Keuls multiple-comparison test. * P < 0.05.

Both plasma total cholesterol and triglyceride concentrations were significantly lower in NZGH than in NZN rats (Fig. 1). By contrast, in AVP-deficient animals, these parameters were not influenced by the presence of hypertension (Fig. 2). Nonetheless, AVP status was influential in that plasma triglyceride concentrations were significantly higher in NZN than in DI/N animals, although the trend was reversed when hypertension was present. Thus, plasma triglycerides were significantly lower in NZGH than in DI/H rats. AVP status had no significant impact on values of plasma total cholesterol for the corresponding hypertensive and normotensive groups.

Plasma corticosterone concentrations were not different between NZGH (82.2 ± 8.0 ng/ml) and NZN (70.4 ± 8.4 ng/ml) rats or between DI/H (55.4 ± 15.1 ng/ml) and DI/N (88.8 ± 16.6 ng/ml) animals. Furthermore, there were no differences in plasma corticosterone between AVP-replete and AVP-deficient animals.

Discussion and Conclusions

The results of the present study highlight two important findings. Firstly, New Zealand genetically hypertensive rats, judged on the basis of similar fasting blood glucose levels, but reduced plasma insulin concentrations, are more insulin-sensitive than normotensive control rats, whether or not they are genetically deficient in the ability to synthesize AVP. Secondly, AVP deficiency is associated with lower fasted plasma insulin concentrations in both the normotensive and hypertensive substrains of rat.

The observation that both of the hypertensive substrains in this study exhibited lower fasted plasma insulin concentrations than their normotensive counterparts is of interest, and adds to the controversy surrounding the relationship between hypertension and insulin resistance in genetic models of hypertension [13,14,15]. However, our finding that the fasted blood glucose concentration is similar in hypertensive and normotensive rats is entirely consistent with the findings of all other publications which we have reviewed on this topic.
The relationship between genetic hypertension and insulin sensitivity has been explored by several groups of workers. There is a number of reports of impaired insulin sensitivity for glucose disposal in anesthetised 
[16,17,18,19] and conscious SHR 
[17,20]. Moreover, under conditions rather similar to those used in our present study (i.e. decapitation, though after a shorter duration of fasting-5 h), elevated plasma insulin concentrations have been demonstrated in Milan hypertensive rats 
[15]. To redress the balance, other workers in studies of conscious SHR have demonstrated either no change in insulin sensitivity 
[21] or that SHR are more insulin-sensitive than WKY rats 
[22,23]. It is worth mentioning that there are also apparent differences in plasma insulin levels between strains of rats. Thus, the plasma insulin concentration was lower in NZN rats here than in WKY rats elsewhere 
[22], but was higher than that reported in Milan normotensive rats 
[15].

Our results in substrains of the NZGH rat, regardless of AVP status, suggest that these animals exhibit enhanced hepatic insulin sensitivity, since the fasted plasma insulin concentration was significantly reduced against the background of an unchanged blood glucose concentration.

Notably, in the current work, fasted plasma insulin concentrations in AVP-deficient rats were significantly lower than in AVP-replete animals, irrespective of the presence of hypertension. This finding further implicates AVP in the regulation of carbohydrate metabolism during response to fasting, a suggestion supported by the observation that AVP stimulates insulin secretion from pancreas in vivo 
[5,7,24] and in vitro 
[25]. However, it should be noted that increments in plasma insulin observed after i.v. injection of AVP in the rat may be due to an indirect action, since both plasma glucagon and glucose increase concomitantly 
[5]. Thus, AVP-stimulated glucagon secretion would result in increased hepatic glucose output and, in turn, increased insulin secretion.

Measurements of plasma glucagon were made in the present study, and although there was a trend for a decrease in DI rats compared with NZ rats, this was only significant in the normotensives. The lowered glucagon in AVP-deficient rats provides compelling evidence that AVP is a physiologically important secretagogue for insulin and glucagon, in agreement with previous reports. The recent demonstration of neurohypophysial hormones stimulating glucagon release from a clonal α-cell line 
[25] further support the notional importance of AVP in carbohydrate metabolism. It is also possible that AVP makes a more direct contribution to hepatic glucose output 
[26]. Our results imply that, in NZGH rats, the liver exhibits an increased sensitivity to glucagon-stimulated glucose output as well as to insulin-stimulated suppression of glycolgenolysis.

Elevated plasma triglyceride levels have been noted in SHR 
[13]. Dahl salt-sensitive rats 
[14] and Milan hypertensive rats 
[15]. Plasma total cholesterol was also raised in the latter study. In the present study, the lower plasma triglycerides and total cholesterol in NZGH compared with normotensive NZN rats are consistent with lowered insulin levels in NZGH, but contrast with the results of the published studies above. The reason for this discrepancy may be due to differences in rat strain and/or to differences in insulin status. It is interesting that, although both total cholesterol and triglyceride concentrations were significantly lower in NZGH rats compared with NZN control rats, this pattern was not repeated in the AVP-deficient rats. This may suggest that, for reasons presently unknown, AVP is required to couple the improvement in carbohydrate metabolism with the improved lipid metabolism in the NZGH rat.

In conclusion, our results are consistent with the notion that AVP may play an important role in glucose metabolism in the rat, though whether this involves pancreatic-dependent or -independent effects remains to be established.

References


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