Tissue noradrenaline and the polyol pathway in experimentally diabetic rats

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- 1 The effects of a six week period of streptozotocin-induced diabetes on tissue catecholamines and on *in vivo* noradrenaline turnover were assessed in rats.
- 2 Noradrenaline concentrations measured in heart ventricle, terminal ileum, vas deferens, spleen and adrenal tissue from the diabetic rats were all found to be elevated compared to those found in control rat tissues. The adrenaline contents of the adrenal glands were also raised in these animals.
- 3 Noradrenaline turnover in heart ventricle, terminal ileum and vas deferens was estimated from the decline in tissue content of the amine following inhibition of its synthesis with α -methyl-*p*-tyrosine. Turnover was found to be increased in all three tissues.
- 4 The involvement of the polyol pathway in the above changes was investigated by examining the effects of continuous treatment with an aldose reductase inhibitor, Statil (ICI 128436) or dietary myo-inositol supplementation. Either treatment was found to prevent or reduce the increases in tissue noradrenaline and in its turnover. Myo-inositol treatment also partially prevented the rise in adrenal adrenaline.
- 5 It is concluded that the elevation of tissue catecholamines and of noradrenaline turnover by diabetes was related to myo-inositol depletion secondary to excessive sorbitol synthesis. Possible mechanisms for the observed increase in noradrenaline turnover could involve Na^+ , K^+ -ATPase depression.

Introduction

Abnormalities of sympathetic nervous function have been reported to occur in diabetic patients (Low *et al.*, 1975). Sympathetic activity in experimentally diabetic rats as assessed by noradrenaline (NA) turnover has been found to be decreased (Yoshida *et al.*, 1987) or increased (Ganguly *et al.*, 1987). The mechanism(s) which underlie these and other diabetes-induced nervous abnormalities remain uncertain. Hypoxia (Low *et al.*, 1986), protein glycosylation (Brownlee *et al.*, 1984) and excessive activity of the aldose reductase pathway (Gabbay, 1975; Kador *et al.*, 1985) have each been proposed.

With the development of effective inhibitors of aldose reductase the possible involvement of the last mentioned has recently received considerable attention (Kador *et al.*, 1985). Aldose reductase inhibition has been shown to prevent and, in some cases,

reverse a number of the changes that are observed in nerves of diabetic animals e.g. reductions in nerve conduction velocity and in the speed of some types of axonal transport (Mayer & Tomlinson, 1983; Mayer *et al.*, 1984). Yoshida *et al.* (1987) reported that the reduced NA turnover which they observed was prevented by aldose reductase inhibition as well as by insulin therapy.

Myo-inositol (MI) concentration in nervous tissue is depressed in short term experimental diabetes, an effect which may be prevented by either aldose reductase inhibition or dietary MI supplementation. This MI depression may mediate some of the above mentioned diabetic-induced changes in nervous function e.g. the reduced nerve conduction velocity and the depression of some types of axonal transport processes (Mayer & Tomlinson, 1983; Mayer *et al.*, 1984).

The present study examines the effects of short term experimental diabetes on tissue catecholamine concentrations and their turnover. The effects of

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either aldose reductase inhibition with Statil (ICI 128436) or dietary MI supplementation on any diabetes induced changes were also investigated.

Methods

Experimental animals

Diabetes was induced in male Wistar rats (200–300 g) by a single injection of 2 ml kg^{-1} of a freshly prepared solution of streptozotocin (20 mg ml^{-1} in pH 4.5 citrate buffer) via a tail vein. Controls received buffer alone. Three days after injection, glucose concentrations were assessed in a drop of blood from a tail vein (obtained by puncturing the vein with a sterile needle) using test strips (BM-test-BG, Boehringer Mannheim). Only rats showing values at or above 22 mm were assigned to diabetic groups. MI-treated rats received 1 g l^{-1} MI in their drinking water from day 3 until the time of death (this resulted in a dose of approximately $1 \text{ g kg}^{-1} \text{ day}^{-1}$).

We have previously found this treatment to prevent sciatic nerve MI depletion (Kofo-Abayomi & Lucas, 1988). Statil (ICI 128,436; 3-(4-bromo-2-fluorobenzyl)-4-oxo-3H-phthalazine-1-yl acetic acid) was administered orally in 5% Tween 80 ($10 \text{ mg Statil ml}^{-1}$) at a dose of $25 \text{ mg kg}^{-1} \text{ day}^{-1}$. All animals not receiving Statil were given equivalent oral doses of 5% Tween 80.

All animals were given free access to food and water.

Tissue catecholamines and blood glucose treatment

Tissue catecholamines were determined by h.p.l.c. with electrochemical detection. The equipment consisted of a pump (Gilson model 302 with manometric module, model 802), an injection valve (Rheodyne model 7125), a reversed phase column ($20 \text{ cm} \times 4 \text{ mm}$ i.d. packed with ODS $5 \mu\text{m}$ hypersil particles), and electrochemical detector (BAS model LC 3A) set at 0.65V and a recorder (Gilson model N1). The mobile phase contained NaH_2PO_4 (0.1 M), NaCl (2 mM), EDTA (0.1 mM) and 10% methanol with octyl-sulphonate (1.2 mM) as the ion-pairing agent.

The solution was degassed under vacuum before use. The flow was 1 ml min^{-1} .

Six weeks after the tail vein injection the rats were weighed, anaesthetized with pentobarbitone (60 mg kg^{-1} , i.p.) and tissues removed and placed in liquid nitrogen. They were subsequently stored at

-20°C until use (usually within 1 month, a period which preliminary studies showed, did not result in significant loss of catecholamines under these conditions). At assay, tissues were homogenized in ice-cold 0.1 M perchloric acid containing EDTA (2.5 mM) and sodium metabisulphite (10 mM) and an amount of epinine approximately equivalent to the expected tissue catecholamine content was added to each tube as an internal standard. The homogenates were then centrifuged at $35,000 g$ for 20 min at 4°C , the supernatants transferred to fresh tubes and the pH raised to 8.6 by the addition of 1 M Tris buffer; 50 mg of acid washed alumina was then added to each tube after which they were shaken vigorously for 20 min in a mechanical shaker. The supernatants were removed and the alumina washed twice with 1 ml distilled deionized water. The catecholamines were finally eluted from the alumina with 0.2 ml 0.1 M perchloric acid. Aliquots ($10 \mu\text{l}$) of this solution were injected onto the column. *In vivo* noradrenaline turnover in heart ventricles, terminal ileum and vas deferens was assessed by measuring the rate of decrease in its concentration in these tissues following the administration of the synthesis inhibitor α -methyl-*p*-tyrosine (AMPT; Spector *et al.*, 1965). The methyl ester of AMPT was dissolved in isotonic saline at a concentration of 60 mg ml^{-1} and 2 ml kg^{-1} of this solution was injected i.p. either 4 or 8 h before the animals were killed. The 8 h groups were reinjected 4 h before they were killed in order to maintain tissue AMPT levels.

NA turnover is expressed as turnover rate constant (h^{-1}). This is the negative regression coefficient with associated standard deviations calculated from the decline of log tissue NA concentration with time (h).

Differences in tissue mass between groups could affect the interpretation of changes in concentrations of NA. Wet tissue weights were, therefore, measured in five additional control, six six-week diabetic rats and in five similarly diabetic rats which received Statil as above.

Glucose concentrations were measured in 0.1 ml samples of blood taken at the time of death by the use of a colourimetric copper reduction method (Asatoor & King, 1954).

Statistics

Differences between the four or three groups in tissue NA concentrations, adrenal adrenaline or in turnover rate constants were assessed initially by one way analysis of variance. Where *P* values of less than 0.05 were obtained, individual means or slopes were compared by the Duncan's method for multiple comparisons; where group sizes differed the harmo-

Table 1 Blood glucose concentrations and growth in control and diabetic rats (SDR) and in diabetic rats treated with myo-inositol (MI) or Statil

	Controls (22)	SDR (24)	SDR + MI (19)	SDR + Statil (20)
Blood glucose (mM)	5.4 ± 2.3	28.9 ± 9.8***	31.8 ± 9.6***	32.8 ± 7.6***
Growth (g)	72.8 ± 17.4	-19.8 ± 37.2***	-24.0 ± 35.7***	-15.6 ± 37.1***

Results are of means ± s.d. with numbers per group in parentheses. The significances of differences from the control values are indicated by: *** $P < 0.001$. No significant differences between the diabetic groups were observed.

nic mean of n was used in the latter test (Bruning & Kintz, 1968). Again P values of < 0.05 were taken to indicate significance.

Results

Diabetes was confirmed in all diabetic groups by their raised blood glucose concentrations and their reduced growth rates (Table 1). These measurements were not significantly changed by treatment with either MI or Statil.

Tissue NA levels were higher in the untreated diabetic group compared to their controls (Table 2). The mean increases were: heart + 95%, spleen + 59%, terminal ileum + 179%, vas deferens + 247% and adrenals + 142%. In each tissue, with the exception of the spleens of the MI-treated group, in which NA was not measured, treatment with MI or Statil appeared to prevent these increases completely, NA levels being similar to control values and significantly lower than those of the untreated diabetic group. Adrenal adrenaline was also increased in the untreated diabetic group by an average of 108% compared to control values.

This elevation was significantly smaller in the MI but not in the Statil-treated group.

NA turnover *in vivo* was increased in the heart ventricles and terminal ilea from the untreated diabetic group compared to values obtained from control rat tissues ($P < 0.001$ and $P < 0.01$ respectively, Table 3). The mean rate constant for NA turnover in the vas deferens was also greater in the untreated diabetic group but the difference was not significant ($P > 0.05$). NA turnover in the heart ventricles and in the ileum of the Statil-treated animals was similar to control values and significantly ($P < 0.001$ and $P < 0.01$ respectively) less than in the untreated diabetic rats.

MI-treatment also appeared to prevent the increased turnover in the terminal ileum in the diabetic animals. A reduced NA turnover rate was observed in the vas deferens of the MI-treated group compared to the untreated diabetic group. The effect of MI on NA turnover in heart ventricles was not assessed. Heart ventricles from the additional diabetic rats weighed approximately 20% less than those from the control animals ($P < 0.01$). In contrast, ilea from these animals were approximately twice the weight of those from controls (Table 3). Statil treatment appeared to have no effect on these changes. No differences between groups were found

Table 2 Noradrenaline turnover (rate constants, h^{-1}) in control and diabetic rats (SDR) and in diabetic rats treated with myo-inositol (MI) or Statil

	Controls (22)	SDR (24)	SDR + MI (19)	SDR + Statil (20)
Heart ventricle	0.059 ± 0.070***	0.123 ± 0.059	ND	0.034 ± 0.210***
Terminal ileum	0.091 ± 0.150**	0.157 ± 0.216	0.064 ± 0.092*	0.088 ± 0.094**
Vas deferens	0.158 ± 0.136	0.223 ± 0.157	0.080 ± 0.083**	0.187 ± 0.112

Results are the negative regression coefficients ± s.d. for the decline of $\log_e$ tissue NA concentrations with time (h). The number of results used to calculate these values are indicated in parentheses. The significances of differences from the untreated diabetic group data: * $P < 0.5$, ** $P < 0.01$ and *** $P < 0.001$.

Table 3. Tissue weights in control and diabetic (SDR) rats and in diabetic rats treated with Statil

	Controls (5)	SDR (6)	SDR + Statil (5)
Heart ventricle (mg)	1000 ± 115	801 ± 116**	788 ± 71**
Spleen (mg)	944 ± 132	759 ± 205	761 ± 172
Ileum (g)	7.43 ± 0.79	14.3 ± 2.8***	16.60 ± 1.15***
Vas deferens (mg)	69.1 ± 4.9	67.0 ± 8.3	68.5 ± 7.7
Adrenals (2) (mg)	63.8 ± 4.5	72.7 ± 7.2	71.1 ± 9.8

Results were obtained in additional animals and are mean ± s.d. The significance of differences from control values are indicated by ** $P < 0.01$ and *** $P < 0.001$.

in the weights of the spleen, vas deferens or adrenal glands.

Discussion

An increase in NA concentrations in various tissues in short-term experimental diabetes has been observed previously in rats (Ganguly *et al.*, 1987; Yoshida *et al.*, 1987) and mice (Garriis, 1987). The present results are in agreement with these reports, but, in addition show, that the diabetes-induced elevation of tissue NA may be related to MI depletion since it was prevented either by MI supplementation or by aldose reductase inhibition. Both have been shown to prevent MI depletion in nerve (Mayer & Tomlinson, 1983).

Changes in tissue weights were observed in the additional control, diabetic and Statil-treated diabetic rats. If the reduction in heart ventricle mass by diabetes was not accompanied by any change in the number of sympathetic neurones then the increase in noradrenaline content per neurone in untreated diabetic rats was approximately 56% rather than the 95% indicated by the tissue NA concentration data of Figure 1. The effects of MI and Statil on NA content per neurone would be correspondingly greater since Statil, and presumably MI, did not prevent the hypoplasia. In contrast, the hyperplasia observed in the ileum means that increases in NA per neurone, again providing the latter remained constant in number, was much greater than that indicated in Figure 1 for the untreated diabetic group. The prevention of the elevation of neuronal NA by MI or Statil would, however be less complete since Statil, and, presumably, MI did not prevent the hyperplasia. The finding of increased catecholamine turnover in tissues of the untreated diabetic animals is in agreement with the study in cardiac muscle reported by Ganguly *et al.* (1987) but not with the

Figure 1 Tissue catecholamine levels in control and diabetic (SDR) rats and in diabetic rats treated with myo-inositol (MI) or Statil. The histograms represent the means and the vertical lines the s.d.'s. Open columns: controls; hatched columns: diabetic rats; solid columns: diabetic rats treated with MI; stippled columns: diabetic rats treated with Statil. Values for the vas deferens, heart ventricles, spleen and terminal ileum are nmol noradrenaline per g of wet tissue while those for the adrenal glands are total noradrenaline or adrenaline content (CA) of both glands. The numbers in each group are given in parentheses and the significance of differences from the untreated diabetic group values are indicated by * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

report by Yoshida *et al.* (1987) who found reduced turnover in heart, brown fat and pancreas. The reason for the disparity between the results of the latter study and those of this work are not clear. The model and duration of diabetes appeared similar. In both studies turnover was assessed from the decline in tissue catecholamines after synthesis inhibition with AMPT.

There are at least two possible mechanisms which could explain an increase in tissue NA turnover in the untreated diabetic group. Na^+ , K^+ -ATPase activity has been reported to be depressed in diabetic nerve, this depression being reversible by either MI or aldose reductase inhibition (Green *et al.*, 1983). Acute inhibition of Na^+ , K^+ -ATPase has been found to increase both acetylcholine and NA turnover (Vizi *et al.*, 1982; Magyar *et al.*, 1986).

Thus, neuronal MI depletion in the untreated diabetic rats may, by depressing Na^+ , K^+ -ATPase, cause the observed increase in NA turnover. Alternatively, hypothyroidism might be involved as Tahiliani & McNeil (1985) have reported depressed plasma levels of both T_3 and T_4 in 6-week streptozotocin-diabetic rats and hypothyroidism has been found to increase cardiac sympathetic activity whether measured by $[^3\text{H}]$ -NA turnover or, as here,

by tissue NA decline following AMPT (Landsberg & Axelrod, 1968). Na^+ , K^+ -ATPase activity has been reported to be depressed by hypothyroidism and increased by T_3 administration (Norgaard *et al.*, 1983). The untreated diabetic rats in the present study may, therefore, have had reduced neuronal Na^+ , K^+ -ATPase activity due to hypothyroidism which resulted in the observed increase in NA turnover. Such a hypothesis does not, however, explain its prevention by MI or Statil unless such treatment prevented the hypothyroidism. The elevation of tissue NA in the absence of AMPT is unlikely to be due to hypothyroidism since Landsberg & Axelrod (1968) found no NA elevation in their hypothyroid rats and Ganguly *et al.* (1987) found no effect of T_3 administration on the elevated tissue NA which they observed in diabetic rats. The latter paper reported a decrease in the proportion of $[^3\text{H}]$ -NA recovered from a cardiac tissue fraction containing the storage vesicles. The possibility of such a storage defect for endogenous NA and, if it occurs, whether it is prevented by aldose reductase inhibition requires investigation.

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