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A comparison between atria from control and streptozotocin-diabetic rats: The effects of dietary myo-inositol

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1 Atria, isolated from control rats, six-week streptozotocin-diabetic rats and from similarly diabetic rats treated with myo-inositol (MI) were compared. The MI treatment was shown to reverse the depressed sciatic nerve MI which was observed in the untreated diabetic group.

2 Spontaneously beating atria from the untreated diabetic animals beat more slowly, and with greater force than tissues from the control group. When electrically driven at 4 Hz they were found to be less sensitive to the negative inotropic effect of acetylcholine. No differences between the two groups were observed in responses to isoprenaline.

3 Intramural nerve stimulation in the presence of 10^{-6}M propranolol (vagal stimulation) had a greater negative inotropic effect in the untreated diabetic rat atria than in the controls. Positive inotropic responses to nerve stimulation in the presence of 10^{-6} M atropine (sympathetic stimulation) were not significantly different between the two groups.

4 Atria from the MI-treated diabetic animals were found to have a lower spontaneous contractile force and greater sensitivity to acetylcholine than tissues from the untreated diabetic animals. The values obtained in both cases were similar to those from the controls. No significant effect of MI treatment on spontaneous contractile rate or on responses to nerve stimulation was demonstrated.

5 Atrial (mainly myocardial) MI was measured in additional control, six-week diabetic and six-week MI-treated diabetic animals. A significantly higher concentration was observed in the MI supplemented group compared to the untreated diabetic group. The mean MI content in the latter group was lower than that obtained from control tissues but not significantly so.

6 The results implicate MI depletion either in the neurones or in the myocardium in at least some of the changes observed. Possible mechanisms involved are discussed.

Introduction

Autonomic neuropathy is a major secondary complication of diabetes (Clark *et al.*, 1979). Defective parasympathetic control of the heart is one of the early detectable changes and may result in persistent tachycardia and loss of beat to beat variation during deep breathing (Wheeler & Watkins, 1973; Lloyd-Mostyn & Watkins, 1975; Feldman, 1981).

A number of studies in experimentally diabetic rats have demonstrated changes in autonomic function, particularly in vagal control of the heart. These changes appear to depend on the period of diabetes. Loss of cholinergic innervation accompanied by supersensitivity to acetylcholine has been found to occur in atria from long-term (8 month) alloxan diabetic rats (Tomlinson & Yusof, 1983). In contrast, cardiac sensitivity to cholinomimetic agents appears to be reduced in shorter term diabetic animals (Foy & Lucas, 1976; Vadlamudi & McNeill, 1983). These changes in sensitivity probably reflect different stages in a developing autonomic neuropathy.

Several mechanisms have been proposed as being responsible for diabetic neuropathy. These include anoxia (Low *et al.*, 1986), protein glycosylation (Vlassara *et al.*, 1981), sorbitol accumulation (Gabbay *et al.*, 1966) and myo-inositol (MI) depletion (Greene & Lattimer, 1985; Hawthorne *et al.*, 1985). According to the last hypothesis MI, which is actively taken up by nervous tissue, is depleted in diabetic nerve because of competition with glucose for membrane transport and by another mechanism related to sorbitol accumula-

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tion. The depletion of MI may, by reducing Na^+/K^+-ATPase activity, further reduce its own uptake and depress other nerve cell functions such as impulse transmission (Gillon *et al.*, 1983) and axonal transport (Mayer & Tomlinson, 1983; Mayer *et al.*, 1984) which may lead to longer term neuropathy. It was therefore decided to investigate the effects of streptozotocin-diabetes with or without dietary MI on autonomic function in isolated atria of the rat.

Methods

Experimental animals

Diabetes was induced in male Wistar rats (200–300 g) by a single injection of 1 ml kg^{-1} of a freshly prepared solution of streptozotocin (40 mg ml^{-1}, 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 (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. Myo-inositol treated rats received 1 g l^{-1} myo-inositol in their drinking water from day 3 until the time of death (this resulted in a dose of approximately 1 g kg^{-1} day^{-1}). At no time were the animals denied access to food or water.

Procedure

Six weeks after the tail vein injections the rats were weighed then killed and their hearts removed and placed in ice-cold physiological saline. Samples of blood (0.1 ml) were taken for glucose estimation by a colorimetric copper reduction method (Asatoor & King, 1954). Both sciatic nerves were removed for myo-inositol assay. Once cardiac contractions had ceased the atria were carefully dissected from the ventricles and remaining non-atrial tissue, and mounted under 1 g tension between two platinum wire electrodes in a 50 ml organ bath containing physiological saline at 37°C. Isometric contractions were measured by means of a force transducer (model UFI, Palmer Bioscience) recording on an oscillograph (model MD2, Palmer Bioscience).

The composition of the physiological saline was (mM): Na^+ 143.3, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.6, Cl^- 128.3, HCO_3^- 24.9, $H_2PO_4^-$ 2.2, SO_4^{2-} 1.2, glucose 5.5, ascorbic acid 0.07 and EDTA 0.067. The solution was bubbled with 95%O_2 and 5%CO_2.

After 1 h incubation, the organ bath contents being changed every 20 min, the force and rate of spontaneous contractions were recorded. The atria were then driven at 4 Hz for the remainder of the experiment by 0.5 ms square wave pulses delivered by a Harvard Research Stimulator. Dose-response curves to isoprenaline and acetylcholine were then obtained. When the tissue had recovered 10^{-6} M propranolol was added to the organ bath and, 10 min later, the driving voltage was increased to 90 V to study the effect of vagal stimulation. The tissue was then washed at 10 min intervals until sensitivity to isoprenaline was restored. Atropine (10^{-1} M) was added to the bath and, 10 min later, the driving voltage was again increased to 90 V to study the effect of sympathetic nerve stimulation. Changes due to vagal or sympathetic stimulation were expressed as % changes in contractile force.

Myo-inositol assay

MI was assessed in the sciatic nerves taken from the above rats and in atria taken from additional animals which had received the same treatment i.e., controls and six-week streptozotocin-diabetic rats with or without MI supplementation. MI was measured by a method similar to that described by Mayer & Tomlinson (1983).

Briefly, tissues were homogenized in 1 ml distilled water containing 30 μg α-methylmannoside as the internal standard. The tubes were then placed in a boiling water bath for 20 min, cooled and deproteinized by the addition of 0.2 ml of 0.2 M $ZnSO_4$ followed 10 min later by 0.2 ml of 0.2 M $Ba(OH)_2$. The tubes were then centrifuged at 800 g for 10 min and the supernatants freeze dried. Trisil (0.5 ml; Pierce, UK) was added to each tube and allowed to stand for 24 h at room temperature before the addition of 2 ml distilled water and 0.2 ml cyclohexane. The tubes were then vortex mixed and samples of the cyclohexane fraction injected into the gas liquid chromatogram (g.l.c. 25 m non-polar column:SGE Bl, with flame ionization detector). Thermal programming was used to enhance the MI peak. External standards containing 30 μg each of α-methylmannoside, sorbitol and MI in 1 ml distilled water were treated in parallel with the tissue homogenate.

Statistics

One-way analysis of variance was carried out on data from the three groups. Where P values of 0.05 or less were obtained differences between individual means were assessed by Duncan's multiple comparisons.

Results

Diabetes was confirmed in both streptozotocin-diabetic groups by their raised blood glucose concentrations and their reduced growth rates. Sciatic nerve MI concentrations were significantly ($P < 0.05$) depressed in the untreated diabetic group compared to

Table 1 Blood glucose concentrations, growth rates, sciatic nerve myo-inositol (MI) concentrations and atrial weights

	Controls (9)	*Diabetic* (9)	*Diabetic + MI* (7)
Blood glucose concentration (mM)	9.9 ± 2.1**	2.2 ± 1.2	20.7 ± 2.6
Growth rate (g day^{-1})	1.8 ± 0.4**	−1.0 ± 0.06	−0.4 ± 0.7
Sciatic nerve MI concentration (μmol g^{-1} wet wt)	4.0 ± 1.1*	2.5 ± 0.8	5.2 ± 1.5**
Weights of atria (mg)	65.4 ± 13.0	64.7 ± 17.5	57.3 ± 11.4

Results are expressed as mean ± s.d. with the numbers per group in parentheses. The significance of differences from the untreated diabetic group are indicated by: *$P<0.05$, **$P>0.01$.

the controls, this depression being prevented by MI administration ($P<0.01$, Table 1).

Spontaneously beating atria from the untreated diabetic animals beat with greater force ($P<0.05$) and lower rate ($P<0.05$) than those from control rats. A comparison between atria from untreated and MI-treated groups indicated that MI treatment prevented the positive inotropic effect of diabetes ($P<0.05$) but had no significant effect on its negative chronotropic effect (Table 2).

A reduced sensitivity to the negative inotropic effect of acetylcholine was observed in atria from the diabetic group. This reduction appeared to be prevented by MI treatment (Figure 1). No significant differences between the three groups in responses to isoprenaline were observed (Figure 2). Responses to vagal stimulation, as assessed by negative inotropic effects of nerve stimulation in the presence of 10^{-6} M propranolol, were greater ($P<0.05$, Table 3) in atria from the untreated diabetic group than in those from the controls. Responses to sympathetic stimulation, as assessed by the positive inotropic effects of nerve stimulation in the presence of 10^{-6} M atropine, were not shown to be changed significantly by diabetes. Similarly MI treatment did not appear to have a significant effect on responses to vagal or sympathetic stimulation. Considerable within group variation was observed in responses to nerve stimulation.

Atrial MI data from the additional rats are presented in Table 4. Although the mean value for the untreated diabetic group was lower than that obtained from the controls, the difference was not significant. The atria from the MI supplemented rats had a significantly ($P<0.01$) higher MI content than did those from untreated diabetic animals.

Discussion

The present data showed that spontaneously beating atria isolated from six-week streptozotocin-diabetic rats beat more slowly, and with greater force than those from controls. Similar changes have been observed previously in atria from 2-week streptozotocin-diabetic rats (Foy & Lucas, 1978). The increase in contractile force appeared to be prevented by myo-inositol supplementation. If this inotropic effect of diabetes is secondary to developing neuropathy then its prevention by dietary myo-inositol would support a role for neural myo-inositol depletion in such neuropathy. The mechanism by which loss of nervous myo-inositol has been proposed to contribute to neuropathy in both human diabetics and experimental animals involves, as mentioned in the introduction, a reduction in the activity of neural Na^+/K^+-ATPase (Green & Lattimer, 1986; Green & Mackay, 1986). A reduction in Na^+/K^+-ATPase activity in diabetic rat myocardial cells, as suggested by Pierce & Dhalla (1983) would provide an alternative explanation for the inotropic effect of diabetes

Table 2 Spontaneously beating atria: rates and forces of contraction

	Controls (9)	*Diabetic* (9)	*Diabetic + MI* (7)
Contractile force (mg)	114 ± 36*	168 ± 42	118 ± 48*
Contractile rate (min^{-1})	240 ± 33*	194 ± 33	204 ± 48

Results are expressed as mean ± s.d. with the numbers per group in parentheses. The significance of differences from the untreated diabetic values are indicated by: *$P<0.05$. MI = myo-inositol.

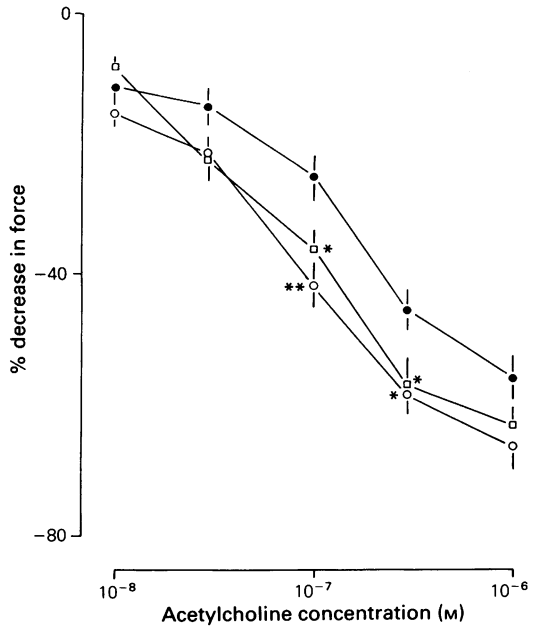

Figure 1 The negative inotropic responses to acetylcholine of atria from control (○), diabetic (●) and 7 myo-inositol-treated diabetic (□) rats. Each point represents the mean of at least 6 values and vertical lines indicate s.e.mean. The significance of differences from the untreated diabetic group values are indicated by $*P<0.05$ and $**P<0.01$.

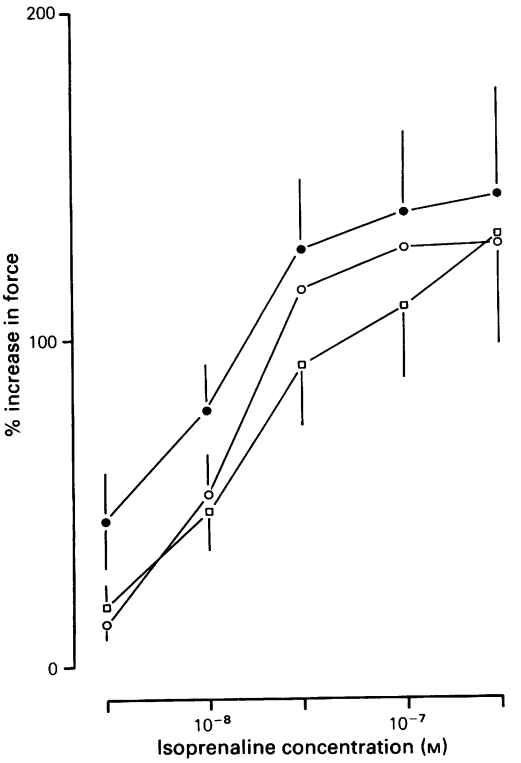

Figure 2 The positive inotropic responses to isoprenaline of atria from control (○), diabetic (●) and myo-inositol-treated diabetic (□) rats. Each point represents the mean of at least 6 values and vertical lines indicate s.e.mean.

in atria. The prevention of the latter by MI administration would, according to this hypothesis, indicate a depletion of MI in diabetic rat atrial tissue which is prevented by oral supplementation and which, as suggested for nervous tissue, leads to reduced Na^+/K^+-ATPase activity. The atrial MI data presented here lend some support to this hypothesis.

The absence of a significant effect of myo-inositol on the reduced spontaneous rate of contraction of diabetic rat atria indicates that this effect is not myo-inositol dependent.

The sensitivity to acetylcholine was significantly lower in atria from the untreated diabetic group. This is in agreement with the results of a study by Vladlamundi & McNeill (1983) who found a reduced sensitivity to carbachol in perfused hearts from 100 day alloxan and streptozotocin diabetic rats. These authors also reported the development of increased responsiveness after longer terms (180–360 days) of

Table 3 The % change in contractile force on increasing the driving voltage to 90 V

	Controls (8)	*Diabetic* (10)	*Diabetic + MI* (8)
Propranolol (10^{-1} M)	− 27.1 ± 7.6*	− 44.4 ± 20.6	− 36.3 ± 18.9
Atropine (10^{-6} M)	21.4 ± 7.9	31.4 ± 21.5	22.1 ± 12.2

Values given are of mean ± s.d. The significance of the difference between control and diabetic values in the presence of propranolol is indicated by: $P<0.05$.

Table 4 Atrial myo-inositol (MI) concentrations in additional control diabetic and myo-inositol-treated diabetic rats

	Atrial MI (μmol g^{-1} wet wt)	
Controls	0.56 ± 0.18	(6)
Diabetic	0.36 ± 0.11	(8)
Diabetic + MI	0.82 ± 0.23**	(5)

Results are mean ± s.d. **Signifies that the myo-inositol treated diabetic group atria had significantly ($P < 0.01$) higher concentrations than the untreated diabetic group.

diabetes. The explanation suggested was that sensitivity might be suppressed at an early stage of neuropathy in compensation for an increased transmitter turnover with later stages of neuropathy involving loss of vagal nerves, accounting for the subsequent supersensitivity. The present data suggest that the mechanism responsible for the temporary reduction in acetylcholine sensitivity is prevented by myo-inositol supplementation. It has been demonstrated that Na^+/K^+-ATPase inhibition increases transmitter turnover (Vizi *et al.*, 1982; Magyar *et al.*, 1986). This raises the possibility that myo-inositol depletion in vagal nerves increases acetylcholine turnover via a reduction in Na^+/K^+-ATPase activity. This increase in transmitter release may then cause a compensatory decrease in myocardial sensitivity. The mechanism by which the atria became less sensitive is not clear. Acute (3 h) exposure of cultured chicken heart cells to high concentrations of muscarinic agonists has been shown to decrease muscarinic receptor number as measured by [^{3}H]-QNB binding (Galper & Smith, 1980). Reduced muscarinic receptor concentrations have been found in ventricles of diabetic rats (Latifpour & McNeill, 1984); however, atrial receptors were not studied, the changes observed were small and were not evident until after 6 months of diabetes. Post-receptor mechanisms could alternatively be involved. The hyperpolarization induced by muscarinic stimulation involves an increased membrane permeability to K^+ accompanied by continued Na^+/K^+-ATPase activity. If the latter were reduced in atrial cells, as suggested earlier, then muscarinic responses might also be expected to be reduced. The prevention of this abnormality by dietary myo-inositol would also be explained if it prevented such a depletion of atrial Na^+/K^+-ATPase activity.

The negative inotropic effect of stimulating nerve endings within the atria in the presence of 10^{-6} M propranolol was greater in tissues from the diabetic group. These results, together with those of a study by Steusse *et al.* (1982), who found increased bradycardia in 7–9 week diabetic rats following vagal stimulation, fit in with the sequence of changes during the development of neuropathy described above with increased vagal activity preceding loss of function at a later stage.

Sympathetic activity, as assessed by electrical stimulation in the presence of atropine, may have been similarly affected, although the results obtained were too variable for this to be clear. Similarly myo-inositol was not demonstrated to have an effect on either vagal or sympathetic function in the present study.

The possibility exists that some of the differences between atria from control and streptozotocin-diabetic groups are due to a direct effect of streptozotocin rather than the resultant diabetes. However, where both streptozotocin and alloxan-diabetic animals have been used in the same study, similar changes in atrial function have been observed (Foy & Lucas, 1978; Valdlamundi & McNeill, 1983). In addition, since tissue MI depletion is a diabetes-dependent effect, MI reversible changes in function result from diabetes rather than a diabetes-independent effect of streptozotocin.

The possible involvement of reductions in Na^+/K^+-ATPase activity, secondary to MI depletion, in some of the changes observed in the untreated diabetic rat atria used in this study requires further investigation. The measurement of neuronal Na^+/K^+-ATPase activity in atrial tissues presents problems since most atrial Na^+/K^+-ATPase activity would be of myocardial origin. However, the measurement of atrial i.e., myocardial Na^+/K^+-ATPase activity would be of interest in view of the possible involvement of decreases in such activity contributing to some of the changes observed in this study. This is, at present, under investigation.

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