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# Comparison between spontaneously beating atria from control and streptozocin-diabetic rats

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Isolated spontaneously beating atria from streptozocin diabetic rats were compared with those from controls. Diabetic atria were found to have reduced rates, increased forces of contraction and reduced sensitivity to the inotropic effects of noradrenaline, isoprenaline, tyramine and calcium. Positive chronotropic responses to tyramine were also reduced but those to noradrenaline and isoprenaline were increased suggesting that tyramine releasable stores of noradrenaline were reduced. Elevation of glucose concentration in the medium from 5·6 to 27 mM resulted in a decrease of inotropic sensitivity to the agents used in both control and diabetic rat atria. Resting contractile force of control rat atria was reduced by the inclusion of either 22 mM 2-deoxyglucose, $10^{-3}$ i.u. insulin $ml^{-1}$ or 5 mM acetate in the medium. The rate was also reduced by medium containing 2-deoxyglucose but increased by insulin. 2-Deoxyglucose also reduced inotropic but increased chronotropic sensitivity to isoprenaline. Possible mechanisms responsible for the changes observed are discussed.

Reduced autonomic control of the cardiovascular system in diabetes mellitus has been suggested by several clinical studies (Wheeler & Watkins, 1973; Bennett, Hosking & Hampton, 1975). These changes have been attributed to autonomic neuropathy (Editorial, 1974).

Recently increased pulse pressure, decreased heart rate and reduced cardiac sensitivity to exogenous catecholamines have been demonstrated in the diabetic pithed rat (Foy & Lucas, 1976). The present report shows the effect of streptozocin diabetes on the rate and force of contraction of atria *in vitro*. Their sensitivity to various agonists has also been examined. Some comparisons have been made between the effects of diabetes and the effects of agents likely to modify the metabolism of heart muscle including glucose, insulin, 2-deoxyglucose (which impairs glucose metabolism by inhibiting the isomerase reaction, Kipnis & Cori, 1959; Brown, 1962), 3-*O*-methyl glucose (a non-metabolizable glucose derivative, Battaglia & Randle, 1960) and acetate, which causes a marked displacement of glucose metabolism (McAllister, Allison & Randle, 1973).

## MATERIALS AND METHODS

### *Streptozocin diabetic rats*

Male CFE rats, 200–300 g, received 1 ml $kg^{-1}$ of a freshly prepared solution of streptozocin (60 $mg^{-1}$ ml pH 4·5 0·1 M citrate buffer) or buffer alone via a tail vein. The latter served as controls. All rats had free access to food and water for two weeks.

### *The isolated atria*

Two weeks after the injections the rats were killed by a blow on the head and their hearts quickly removed and placed in ice cold physiological saline. After contractions had ceased, the atria were carefully dissected from the ventricles and remaining non-atrial tissue and one atrium connected to a tissue holder and the other to a tension transducer recording on a 'Devices' two channel recorder, the preparation being set up in a 10 ml organ bath containing physiological saline at 34°. The atria resumed beating almost immediately and the baseline was adjusted to 300 mg. Immediately after the removal of the heart a 0·1 ml blood sample was taken from the inferior vena cava for blood glucose assay according to Asatoor & King (1954). Only rats with blood glucose concentrations greater than 300 mg/100 ml were included in the diabetic groups: only 3 rats that had received streptozocin failed to meet this requirement.

The composition of the physiological saline used, unless otherwise indicated, was (mM): NaCl 117; KCl 4·7; $CaCl_2 6H_2O$ 2·5; $KH_2PO_4$ 1·2; $MgSO_4.7H_2O$ 1·2; $NaHCO_3$ 22·3 and dextrose 5·6. The solution was gassed with a mixture of 5% $CO_2$ in oxygen.

Where control and diabetic atria were compared both were incubated simultaneously in the same organ bath.

After 1 h incubation the force and rate of atrial contractions were measured. The following drugs

* Correspondence.

were then added to the organ bath at the concentrations indicated: noradrenaline 25, 50 and 100 ng $ml^{-1}$; isoprenaline 5, 10 and 25 ng $ml^{-1}$; tyramine 0·5, 1 and 2 $\mu g\ ml^{-1}$; calcium 0·625, 1·25 and 2·5 mM.

Their order of addition was randomized and the drug was left in contact with the atria until the maximum response had been obtained. The frequency of addition was not greater than once every 5 min. Additional experiments were made to investigate the effects of altered medium composition on the atria. To the standard medium, one of the following compounds was added different atria being used for each solution:

D-glucose (22 mM); 2-deoxyglucose (22 mM); 3-*O*-methylglucose (22 mM); insulin ($10^{-3}$ i.u. $ml^{-1}$); acetate (5 mM acetic acid buffered to pH 7·4 with sodium hydroxide before addition).

All measurements of changes in auricular contractile force were expressed as percentage changes. This was to avoid variations between auricles due to differences in the proportion of atrial muscle from which contractions could be recorded, i.e. the proportion of muscle between the attachment leading to the transducer and that leading to the tissue holder. A statistically significant difference was assumed where $P$ values 0·05 or less were obtained, using a Student's $t$-test.

## RESULTS

*Comparison between diabetic and control rat atria*

Atrial weights were similar in the two groups (Table 1). Pre-dose contractile force was greater and the rate lower in the atria isolated from the diabetic rats compared with those of the controls (Table 1). Inotropic responses to noradrenaline, isoprenaline, tyramine and calcium were all reduced in the diabetic rat atria (Figs 1 and 2). Chronotropic responses to tyramine were also reduced but those to noradrenaline and isoprenaline were, in contrast, slightly increased (Figs 1 and 2).

Table 1. *The effects of diabetes on the contractile force, contractile rate and weight of isolated auricles.*

| | Controls | Diabetic |
|---|---|---|
| Contractile force (mg) | 213·1 ± 22·1 (21) | 317·4 ± 39·1 (21)* |
| Contractile rate ($min^{-1}$) | 266·5 ± 12·3 (21) | 201·3 ± 10·3 (21)*** |
| Auricle wt (g) | 0·057 ± 0·003 (11) | 0·061 ± 0·002 (9) |

The numbers per group are indicated in parentheses. Asterisks indicate significance of difference from controls * $P < 0·05$; *** $P < 0·001$.

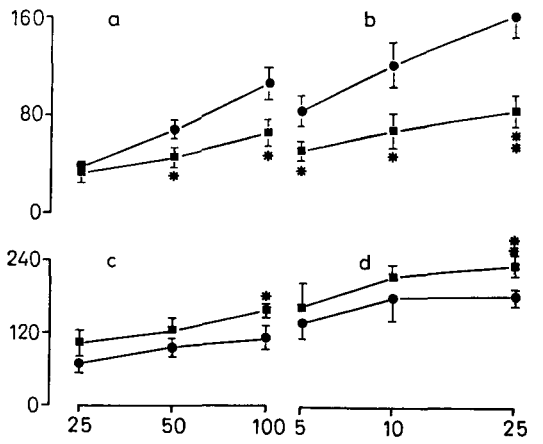


FIG. 1. Inotropic and chronotropic responses to noradrenaline (a, c) and isoprenaline (b, d) of auricles isolated from control (●, n = 8) and two week streptozocin (60 mg $kg^{-1}$) diabetic (■, n = 6) rats. Vertical lines show s.e.m. Significant differences from controls are indicated by * $P < 0·05$ and ** $P < 0·01$. Ordinates: (a, b) Increase in force (%); (c, d) Increase in rate $min^{-1}$. Abscissa: ng $ml^{-1}$.

*Effects of excess glucose on diabetic and control rat atria*

Before the addition of drugs, neither the force nor the rate of contraction were significantly changed in either diabetic or control atria by increasing the

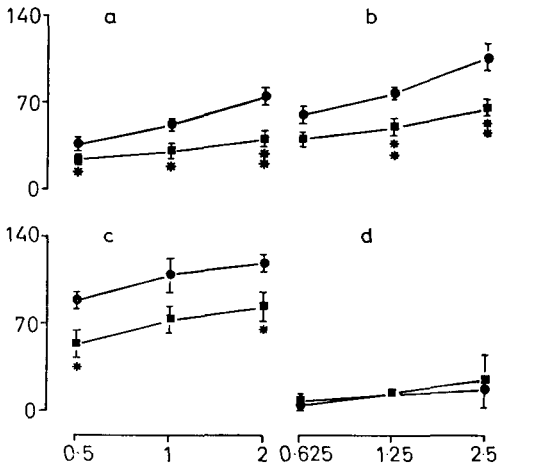


FIG. 2. Inotropic and chronotropic responses to tyramine (a, c) and calcium (b, d) of auricles isolated from control (●, n = 8) and two week streptozocin (60 mg $kg^{-1}$) diabetic (■, n = 6) rats. Vertical lines show s.e.m. Significant differences from controls are indicated by * $P < 0·05$ and ** $P < 0·01$. Ordinates: as for Fig. 1. Abscissae: (a, c) $\mu g\ ml^{-1}$; (b, d) excess $Ca^{2+}$ (mM).

glucose in the medium from 5·6 to 27·8 mM (Table 2). Similarly, the chronotropic responses to isoprenaline were not changed in either group (Fig. 3). Positive inotropic responses to isoprenaline were, however, reduced in both groups (Fig. 3).

Table 2. *The effects of raised medium glucose on the contractile force and rate of control and diabetic rat atria and of* 3-O-*methylglucose* (3-*OMG*), 2-*deoxyglucose* (2-DG), *insulin and acetate on those of control rat atria.*

| Group | Medium includes | Force (mg) | Rate min$^{-1}$ |
|---|---|---|---|
| Control rat atria | 5·6 mM glucose | 213·1 ± 22·1 (21) | 266·5 ± 12·3 (21) |
| | 27·8 mM glucose | 194·5 ± 35·0 (9) | 295·2 ± 18·6 (9) |
| Diabetic rat atria | 5·6 mM glucose | 317·4 ± 39·1 (21) | 201·3 ± 10·3 (21) |
| | 27·8 mM glucose | 334·9 ± 30·9 (5) | 241·4 ± 44·2 (5) |
| Control rat atria | 5·6 mM glucose | 291·8 ± 18·4 (16) | 295·1 ± 8·3 (16) |
| | +22 mM 3-OMG | 236·3 ± 40·1 (12) | 297·9 ± 10·6 (12) |
| | +22 mM 2-DG | 212·4 ± 16·2 (16)*** | 237·6 ± 6·8 (16)*** |
| | +$10^{-3}$ i.u. insulin ml$^{-1}$ | 254·1 ± 17·0 (8)* | 346·9 ± 12·2 (8)** |
| Control rat atria | 5·6 mM glucose | 260·0 ± 17·5 (8) | 264·2 ± 9·8 (8) |
| | +5 mM acetate | 209·1 ± 16·5 (8)** | 257·1 ± 8·3 (8) |

Numbers per group are indicated in parentheses.
Asterisks indicate significance of differences from atria incubated in control medium. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

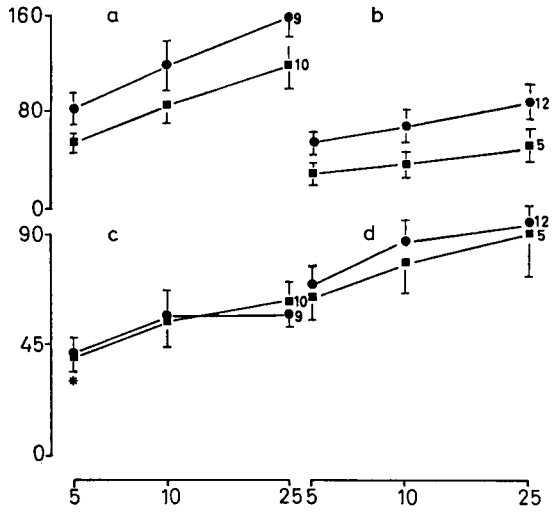


FIG. 3. Effects of elevation of glucose in the medium from 5·6 to 27·8 mM on the positive inotropic and chronotropic responses of control (a, c) and diabetic (b, d) rat atria to isoprenaline. Each point represents the mean of n observations indicated at the end of each graph. Vertical lines show s.e.m. Significant differences between responses in high (■) and low (●) glucose medium are indicated by * $P < 0.05$. Ordinates: as for Fig. 1. Abscissa: Isoprenaline ($\mu$g ml$^{-1}$).

*Effects on control rat atria of adding* 3-O-*methyl, 2-deoxyglucose, insulin or acetate to the medium*

The effects of the additives on contractile force and rate are illustrated in Table 2. Contractile force as reduced by 2-deoxyglucose, acetate and insulin. 3-*O*-Methylglucose had no significant effect. The rate was reduced in the medium containing 2-deoxyglucose but was increased in that containing insulin. Neither 3-*O*-methylglucose nor acetate significantly changed the rate.

The effects of 3-*O*-methylglucose, 2-deoxyglucose and insulin on the positive inotropic and chronotropic responses to isoprenaline are illustrated in Fig. 4. The auricles incubated in the presence of 2-dexoyglucose had greatly reduced positive inotropic and slightly increased positive chronotropic responses to isoprenaline. Neither insulin nor 3-*O*-methylglucose significantly changed isoprenaline sensitivity. The atria incubated in the presence of acetate did not respond differently to tyramine or noradrenaline from those incubated in control medium.

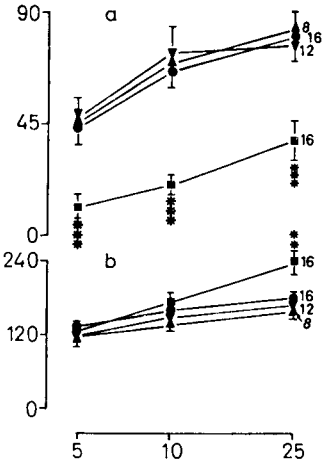


FIG. 4. Effects of 3-*O*-methylglucose, 2-deoxyglucose and or insulin in the medium on the positive inotropic and chronotropic responses of control rat atria to isoprenaline. Each point represents the mean of n observations indicated at the end of each graph. Vertical lines show s.e.m. (●) control medium, (▼) + 22 mM 3-*O*-methylglucose, (▲) + $10^{-3}$ i.u. ml$^{-1}$ insulin and (■) + 22 mM 2-deoxyglucose. Significant differences between responses in test and control medium are indicated by ** $P < 0.01$ and *** $P < 0.0001$. Ordinates: (a) increase in force (%) (b) = increase in rate, min$^{-1}$. Abscissa: Isoprenaline (ng ml$^{-1}$).

## DISCUSSION

The decreased contractile rate of atria from two week streptozocin diabetic rats may be analogous to the decreased heart rate previously observed in similarly diabetic rats when pithed (Foy & Lucas, 1976). Since the inclusion of insulin in the incubation medium of control rat atria increased their contractile rate, the decreased rate of the diabetic rat atria may have been due to their characteristic hypoinsulinaemia (Rerup, 1969). Insulin increases glucose metabolism in the myocardium (Chain, Mansford & Opie, 1969). The possibility that decreased glucose metabolism in diabetic rat atria was responsible for their decreased rate of contraction must, therefore, be considered. 2-Deoxyglucose inhibits glucose metabolism. It has been shown to reduce the rates of contraction of isolated atrial cells (Lacuara & Lacuara, 1973) and this effect was attributed to ATP depletion secondary to decreased glucose metabolism. Our findings are in agreement with this. The reduced contractile rates of the diabetic rat atria may, therefore, be the result of ATP depletion due to depressed glucose metabolism secondary to hypoinsulinaemia. Acetate was expected to cause a major displacement of glucose metabolism. But its lack of effect on contractile rate, suggests that, on this parameter, acetate metabolism compensated for any potential effects of decreased glucose metabolism. Reduced heart rate responses to isoprenaline have previously been demonstrated in streptozocin diabetic rats (Foy & Lucas, 1976). The mechanisms involved cannot be deduced from the present experiments since atria from diabetic rats showed slightly increased, rather than decreased, chronotropic responses to catecholamines. The decreased response to tyramine may indicate that tyramine-releasable stores of noradrenaline were reduced in atria from diabetic rats. Reduced cardiac noradrenaline stores have been found in the human diabetic (Neubauer & Christensen, 1976).

The increased contractile force of atria from streptozocin diabetic rats may provide an explanation for the increased pulse pressures observed in pithed diabetic rats (Foy & Lucas, 1976) but such an explanation also requires a demonstration that the ventricular myocardium is similarly affected.

The mechanisms involved in the increased force of contraction shown by atria from diabetic rats are unclear. Inhibition of glucose metabolism does not explain this observation since 2-deoxyglucose and acetate decreased the force of contraction of normal atria. Moreover, addition of insulin, which would be expected to increase myocardial glucose metabolism, decreased the contractile force of normal atria. Elevation of cardiac cyclic (c) AMP is said to be responsible for the positive inotropic responses to catecholamines (Wollenberger, 1975). The increased contractile force of the streptozocin diabetic rat atria may, therefore be due to higher resting concentrations of AMP. Hypoinsulinaemia and hyperglucagonaemia (Pagliora, Stillings & others, 1975) which occur in the diabetic rat might both be expected to increase cardiac cAMP concentrations. This explanation may well fit in with the reduction in force of contraction produced by insulin we observed in normal atria, since insulin has been shown to increase cAMP phosphodiesterase concentrations in the rat heart (Das, 1973).

The inotropic responses to the various agents used were expressed as percentages of the resting contractile force. The reduced inotropic responses of the diabetic rat atria were therefore, at least in part, due to their higher resting contractile force. It is also possible that in the absence of inotropic agents, the forces of contraction of the diabetic atria were closer to their maxima. Further increases under the influence of such agents may, for this reason, be reduced.

The reduction in both resting contractile force and inotropic response to isoprenaline by 2-deoxyglucose may be due to diminished glucose metabolism. It is more difficult to explain the reduced inotropic responses of both control and diabetic rat atria in the presence of increased glucose concentrations since similarly increased glucose concentrations have been shown to enhance glucose metabolism (Chain & others, 1969). Increased osmolarity is unlikely to be responsible since an equimolar addition of 3-*O*-methylglucose had no such effect. It would appear that if a similar effect occurred in ventricular muscle then the increased plasma glucose concentration of diabetic rats would contribute to their reduced pulse pressure responses to isoprenaline which have previously been demonstrated (Foy & Lucas, 1976).

The relevance of these observations to clinical diabetes mellitus is uncertain since no change in heart rate or pulse pressure has been found in diabetics (Christlieb, Janka & others, 1976). No work appears to have been done to examine the responsiveness of the human diabetic heart to catecholamines. If similar reductions in inotropic sensitivities could be demonstrated then this, in addition to autonomic neuropathy, might contribute to a reduced autonomic control of the heart.

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