Sex-dependent effect of streptozotocin-induced diabetes mellitus on hepatic steroid metabolism in the rat

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Abstract. Diabetes mellitus is known to affect drug and steroid metabolism in the rat liver. Recently it has been shown that the effect on drug metabolism is both transient and sex-dependent. This study shows that the effect of diabetes on steroid metabolism is also sex-dependent i.e. only seen in the male and the effect is always to abolish the sex differences in steroid metabolism found in the intact animals. 7α-hydroxylase activity, which is higher in the female, is increased by diabetes in the male whereas 6β-hydroxylase, 16α-hydroxylase and 17-oxosteroid reductase, which are all higher in the male, are decreased by diabetes. This is a very similar result to that found for drug metabolism and indicates that insulin plays a role in the maintenance of sex differences in hepatic steroid metabolism in the rat as it does for drug metabolism.

Diabetes mellitus is a disorder characterised by hyperglycaemia, hyperlipidaemia, ketosis and resultant degenerative changes. The disorder is caused either by the lack of insulin secretion by the pancreatic B-cells (type I or insulin-dependent diabetes) or lack of responsiveness to insulin by peripheral tissues (type II or non-insulin-dependent diabetes).

Streptozotocin (STZ) is a nitrosourea derivative of 2-deoxyglucose and is thought to be a selective B-cell toxin (Like & Rossini 1976; Rossini et al. 1977) and, thus, STZ-treated animals are thought to be a good model for type I diabetes. A number of reports have indicated that STZ-induced diabetes mellitus can affect hepatic steroid metabolism in the rat. Leaming et al. (1982) showed that conversion of dehydroepiandrosterone to androst-4-ene-3,17-dione and testosterone was enhanced in STZ-treated animals and De Hertogh et al. (1984) indicated that oestradiol metabolism was increased in similarly treated animals. At a more specified enzyme level cholesterol 7α-hydroxylase activity is enhanced by STZ treatment (Subbiah & Yunker 1984) whereas testosterone 5α-reductase is unaffected by STZ (Warren et al. 1983).

Steroid and drug metabolism by the rat liver is known to be sex-dependent (Kato 1974; Gustafsson et al. 1980; Colby 1980) and the effects of diabetes mellitus have been shown also to be sex-dependent with regard to drug metabolism (Dixon et al. 1961; Kato & Gillette 1965; Skett & Joels 1984). In all cases it is the enzyme activities that are higher in the male that are affected most and then only in the male animal. As steroid metabolism is intimately linked to drug metabolism in the rat liver by the enzymes involved and by an apparent mutual control mechanism (Skett et al. 1984), it was of interest to study if the effect of STZ-induced diabetes mellitus was sex-dependent in the rat liver with regard to steroid metabolism.

Materials and Methods

Animals
Mature male and female animals of inbred Wistar stock, reared in the department, were used throughout the experiment. Males were 250–300 g and females 225–250 g at the start of the experiment. Animals were kept in wire-bottomed cages and allowed free access to food (CRM Nuts, Labsure, Croydon) and tap water through-
out the experiment. Animal room temperature was maintained at 19 ± 1°C and light were on from 08.00–20.00 h. Animals were made diabetic by a single iv injection of streptozotocin (60 mg/kg in distilled water—made up immediately prior to use) given under halothane/N₂O anaesthesia 3 days prior to sacrifice as previously described (Skott & Joels 1984). Controls were anaesthetised and given vehicle only. Where appropriate, insulin (50 U/kg; Neulente Insulin Zinc Suspension BP, Wellcome, Welwyn Garden City) was given directly after the streptozotocin and at 24 h intervals until sacrifice.

Chemicals
Streptozotocin and trisodium isocitrate were obtained from Sigma Chemical Co. Ltd., Poole, Dorset. Isocitrate dehydrogenase and NADP⁺ were purchased from Boehringer Mannheim Ltd., Lewes, Sussex. [4-¹⁴C]androst-4-ene-3,17-dione was supplied by Amersham International p.l.c., Amersham, Bucks.

Preparation of microsomes and serum
At the end of the experiment, the animals were killed by CO₂-asphyxiation and cervical dislocation. Blood was collected from the cut neck vessels and allowed to clot at 4°C. Serum was removed after centrifugation of the coagulated blood at 2500 x g for 20 min in a Damon/IEC DPR-6000 refrigerated centrifuge. Serum was stored at −20°C until required for assay. All assays were performed within 2 weeks of serum preparation.

The liver was removed from the animal as soon as possible and rinsed in ice-cold Tris/sucrose buffer (0.25 M sucrose/0.1 M Tris, pH 7.4) to remove excess blood and other foreign material. The liver was finely chopped and homogenised in 4 vols of buffer using a Potter-Elvehjem homogeniser with loose-fitting Teflon pestle. A microsomal preparation was made by the Ca²⁺-precipitation method of Cinti et al. (1972). The homogenate was centrifuged at 12500 x g for 10 min in an MSE HS-21 refrigerated centrifuge at 4°C. The supernatant was removed and 1 M calcium chloride solution added to make the final concentration 8 μM. The mixture was left on ice for 5 min and subsequently centrifuged at 21000 x g for 20 min at 4°C. The resulting pellet was collected and resuspended in 12 ml of buffer by gentle homogenisation. The suspension produced is referred to as the microsomal preparation. The microsomes were stored on ice and used the same day for all assays.

Assay of enzyme activity
The enzyme activity in the microsomal preparation was assayed using androst-4-ene-3,17-dione as substrate. The method was essentially that of Berg & Gustafsson (1973) and involved incubating the microsomal preparation with [¹⁴C]-labelled substrate in the presence of an NADPH-regenerating system. The metabolites and unused substrate were extracted into chloroform/methanol (2:1) and separated by thin-layer chromatography. Metabolites have previously been identified by gas chromatography-mass spectrometry using authentic standards (Berg & Gustafsson 1973). The radio-labelled bands were located by autoradiography and scraped into Packard ES-299 scintillant. Radioactivity in each sample was measured in a Packard Tri-carb scintillation counter and enzyme activity calculated using a custom-made computer program.

Other assays
Serum glucose was assayed by a technique based on the glucose oxidase method (Carroll et al. 1970) using a kit supplied by Sigma Chemical Co. Ltd.

Protein content of the microsomal preparation was measured using the method of Lowry et al. (1951). Bovine serum albumin was used as standard in this assay.

Serum triglycerides were assayed by the method of Fletcher (1968), which involves hydrolysis of the lipids to yield glycerol and subsequent oxidation to give formaldehyde. The formaldehyde was assayed by the method of Nash (1953).

| Table 1. |
| The effects of streptozotocin and insulin treatment on the serum glucose, ketone and triglyceride concentrations in male and female rats. |

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>Serum glucose (mM)</th>
<th>Serum ketones</th>
<th>Serum triglycerides (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>male</td>
<td>6.58 ± 0.52</td>
<td>absence</td>
<td>1.54 ± 0.42</td>
</tr>
<tr>
<td>Streptozotocin</td>
<td>male</td>
<td>29.12 ± 0.79*</td>
<td>presence</td>
<td>9.75 ± 1.71*</td>
</tr>
<tr>
<td>Streptozotocin + insulin</td>
<td>male</td>
<td>4.48 ± 2.98</td>
<td>absence</td>
<td>2.54 ± 0.78</td>
</tr>
<tr>
<td>Control</td>
<td>female</td>
<td>5.70 ± 0.78</td>
<td>absence</td>
<td>1.78 ± 0.59</td>
</tr>
<tr>
<td>Streptozotocin</td>
<td>female</td>
<td>27.35 ± 4.45*</td>
<td>presence</td>
<td>8.48 ± 3.62*</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD of 5 animals. * Groups are significantly different from groups unstarred.
The effect of streptozotocin-induced diabetes mellitus and replacement treatment with insulin on androst-4-ene-3,17-dione metabolism in male and female rats. Groups are as follows: A, control males; B, STZ-treated males; C, STZ-treated males + insulin; D, control females; E, STZ-treated females. 7α, 6β and 16α refer to the hydroxylase activities, 5α to the reductase activity and 17-OHSD is the 17-oxosteroid reductase activity. The letters above each group of results are the groups arranged in rank order and those groups underlined are not significantly different.

Results are expressed as mean ± sd of 5 animals.

Statistics

Five animals were used in each group and group means, standard deviations and variances calculated using a custom-made computer program. Means were compared using Duncan's multiple range test and the level of significance set at P < 0.05.

Results

Table 1 shows the serum parameters to assess the degree of diabetes mellitus achieved by the STZ-treatment and the success of the insulin treatment. It is seen that the STZ-treated animals exhibit a very much increased serum glucose level (4.4 × in the male and 5.5 × in the female) and this is restored to within the normal range by insulin treatment in the male. Serum ketones are only detected in the STZ-treated animals and serum triglycerides are significantly increased in STZ-treated animals and are restored to the normal level by insulin treatment in the male. The STZ-treated animals are, therefore, hyperglycaemic, hyperlipidaemic and ketotic – all signs of severe diabetes mellitus.

Incubation of androst-4-ene-3,17-dione with hepatic microsomal preparations and subsequent separation and quantitation of the metabolites as described above allows the calculation of the following enzyme activities: 7α-, 6β- and 16α-hydroxylases, 17-oxosteroid reductase (no attempt was made to separate the 17α- and 17β-components) and 5α-reductase. Fig. 1 shows the effects of streptozotocin (STZ)-induced diabetes mellitus and subsequent treatment of the diabetic state with insulin on the activities of the above mentioned enzyme activities.

The 7α-hydroxylase activity is increased after STZ-treatment in the male but is unaffected by the same treatment in the female. Insulin reverses the
effect of STZ in the male. It is interesting to note that the enzyme activity in the STZ-treated male is similar to that in the female i.e. STZ-treatment abolishes the sex difference in enzyme activity by moving the activity in the male towards that of the female.

The 6β-hydroxylase activity is significantly reduced following STZ-treatment in the male and is restored to normal levels by treatment with insulin. As with the 7α-hydroxylase, no effect is seen in the female. The 16α-hydroxylase activity shows a very similar pattern to the 6β-hydroxylase although more pronounced. The high male activity is reduced by STZ-treatment to a level indistinguishable from the female. Insulin treatment reverses the effect of the STZ and restores a control male activity.

The 17-oxosteroid reductase activity is also reduced following STZ-treatment in the male and is increased again after insulin treatment. In the female the lower 17-oxosteroid reductase activity is unaffected by STZ-treatment. As with all of the enzymes so far discussed STZ-treatment abolishes the sex difference in activity by altering the male to the female level of enzyme.

The 5α-reductase activity is unaffected by STZ-treatment in both the male and female animals and is also unaffected by insulin treatment of STZ-treated males. There is, however, a marked sex difference in the activity of the 5α-reductase — the female being over 3 times higher.

Discussion

Streptozotocin, and the alternative B-cell toxin, alloxan, have been used previously to assess the effects of diabetes mellitus on hepatic function, particularly as regards lipid metabolism (Van Harken et al. 1969; Nepokroeff et al. 1974; Raghu-pathy et al. 1975; Whiting et al. 1977; Dang et al. 1984a,b). The effects of B-cell toxins on drug metabolism in liver have also been well studied. There have been reports of increased metabolism of aniline in diabetic animals (Past & Cook 1982) as well as markedly decreased activity for many other substrates (Dixon et al. 1961; Kato & Gillette 1965; Remke et al. 1978).

In this study it was shown that STZ-induced diabetes mellitus causes a change in enzyme activity which is dependent on the enzyme being studied. The 7α-hydroxylase activity is increased, the 6β- and 16α-hydroxylases and the 5α-reductase activity unaffected by STZ-treatment. The 17-oxosteroid reductase activity is decreased in a similar manner to the 6β-hydroxylase. It is interesting to note that these changes were only seen in the male animal — the female being unaffected by STZ-treatment. Furthermore, the enzyme activities affected in the male were always sex-dependent (cf. male and female control activities) and always moved towards the female level of enzyme activity. All of the effects seen after STZ were reversed by insulin treatment indicating that STZ is exerting its effects via its diabetogenic action and not as a result of a direct toxic action on the liver. This is in complete agreement with earlier work on hepatic drug metabolism (Skett et al. 1984; Skett & Joels 1984). This correlation with drug metabolism is hardly surprising as the two systems appear to use many of the same enzymes and are thought to be controlled in a very similar manner (Gustafsson et al. 1980; Skett & Gustafsson 1979). The only exception to the move towards a more female type of metabolism is the lack of effect on the 5α-reductase. The reason for this is unknown.

Alterations in steroid metabolism are not unknown in the diabetic state. Peripheral metabolism of oestradiol is enhanced in STZ-induced diabetes mellitus (De Hertog et al. 1984) as is the conversion of dehydroepiandrosterone to testosterone in hepatic cytosol (Leaming et al. 1982). Interestingly, Subbiah & Yunker (1984) have shown that cholesterol 7α-hydroxylase activity is increased in STZ-induced diabetes — this data is in agreement with the results in this study on the 7α-hydroxylase active on androst-4-ene-3, 17-dione. Also, Warren et al. (1983) indicated that STZ-treatment was ineffective in altering testosterone 5α-reductase activity in hepatic microsomes, a result strikingly similar to this study.

Streptozotocin-induced diabetes mellitus has, therefore, a substrate- and sex-dependent effect on hepatic steroid metabolism in the rat. The effects are very similar to those seen for drug metabolism and are probably related to the hormonal changes known to influence drug and steroid metabolism in the rat liver (Gustafsson et al. 1980; Skett et al. 1984). This work gives further proof to the common control of drug and steroid metabolism in the rat and indicates that insulin plays a role in the maintenance of sex differences in hepatic steroid metabolism in the rat.
Acknowledgment

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References


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