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EARLY EFFECTS OF THE ADMINISTRATION OF STREPTOZOTOCIN ON MUSCLE GLYCOGEN

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Abstract—The i.v. administration of streptozotocin (40 or 80 mg/kg body wt) decreases muscle glycogen in fasted rats within a few hours and impairs glycogen accumulation after refeeding. This may help to elucidate the simultaneous increase in plasma glucose levels. The possible role of changes in the level of insulin is discussed.

It is known that 2–4 hr after streptozotocin administration blood glucose levels are remarkably increased both in fasted [1] and even more in fed rats [2]. The amplitude of this effect suggests that important changes of sugar metabolism occur simultaneously in some tissues. Little attention has been paid to changes in tissue glycogen: increased levels have been reported only in the liver [3]; surprisingly, we could not find any data concerning muscle.

METHODS

Random groups of male Wistar albino rats, 170 g body wt, fasted for 18 hr, received either streptozotocin (40 or 80 mg/kg body wt in 0·20 ml saline buffered at pH 4·5 with 10 mM citrate), or the vehicle only, by i.v. injection. The early effects of the drug on blood glucose and muscle glycogen could be amplified by giving the animals free access to food 2 hr after the injection.

Groups of animals were decapitated 0, 2, 4, 6 and 8 hr after streptozotocin administration, their blood collected and the extensor digitorum longus and the soleus muscles removed and weighed. Muscle glycogen was then purified and determined colorimetrically [4]. Commercially available kits were used to assay plasma glucose (by the glucose oxidase–peroxidase method) and IR-insulin (using human insulin as the standard).

Streptozotocin was obtained from the Upjohn Co., Kalamazoo, MI, U.S.A. Kits for glucose assays were supplied by Sclavo ISVT (Siena, Italy); those for insulin assay were supplied by the Radiochemical Centre, Amersham, U.K.

RESULTS AND DISCUSSION

In the fasted rats, streptozotocin significantly decreased muscle glycogen ($P < 0.01$, F-test) both in a white (extensor digitorum longus, ELD) and in a red (soleus, S) muscle. Besides the strong dependence on dosage ($P < 0.01$)—which is mainly attributable to the response of the ELD—the quantitative response of the two muscles was different ($P < 0.01$) and the

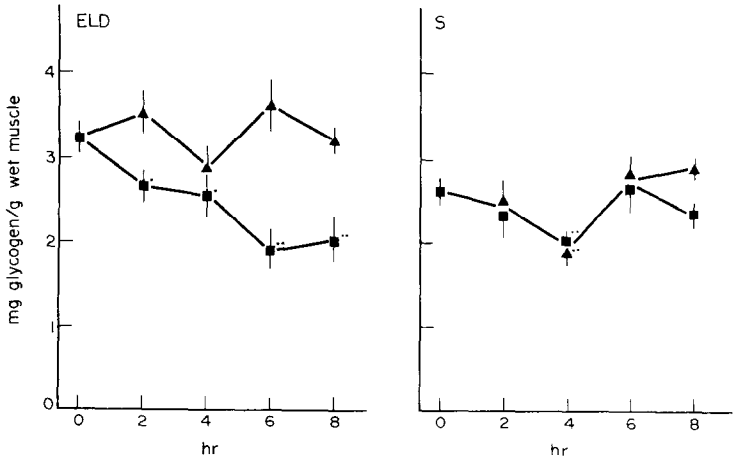

Fig. 1. Effect of the administration of 40 mg/kg body wt (▲) or of 80 mg/kg body wt (■) streptozotocin on glycogen levels in the extensor digitorum longus (ELD) or in the soleus (S) muscles in fasted animals. Ordinate, muscle glycogen levels (mg/g wet tissue); abscissa, hr after the i.v. injection of the drug. Means ± S.E.M. of 5 samples are given. The statistical significance (t-test) is shown according to the following notations: $\cdot P < 0.05$; $\cdot\cdot P < 0.01$. The injection of the vehicle did not effect the levels of muscle glycogen at any time.

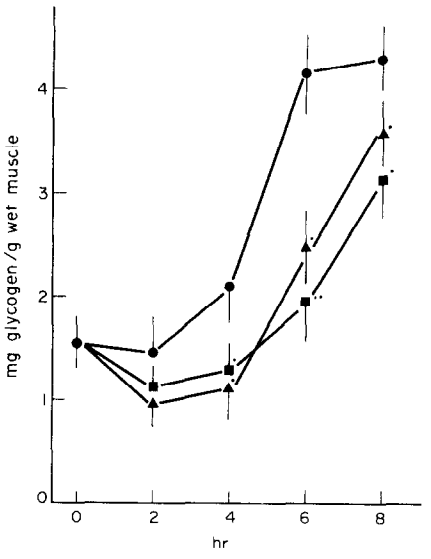

Fig. 2. Effect of the administration of buffered saline (●), 40 mg/kg body wt (▲) or 80 mg/kg body wt (■) streptozotocin on glycogen levels in the ELD muscle. Animals had free access to food 2 hr after the drug injection (i.e. immediately after the sacrifice of the first experimental group). Ordinate, mg glycogen/g wet tissue; abscissa, hr after the i.v. injection of the drug. Means ± S.E.M. of 6 samples are given. Statistical significance is shown as in Fig. 1.

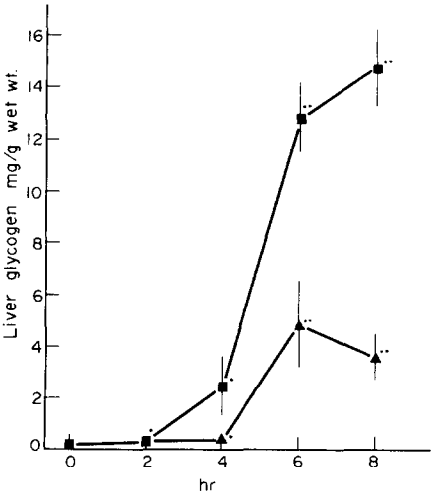

Fig. 3. Effect of the administration of 40 mg/kg (▲) or 80 mg/kg body wt (■) streptozotocin on the levels of liver glycogen in fasted rats. Means ± S.E.M. of 5 samples are given. Statistical significance is shown as in Fig. 1.

pattern of glycogen changes in the two muscles differed as a function of dose and time ($P < 0.05$). These variations may be consequences of the different metabolic attitudes of white and red muscle fibres (see Fig. 1).

After refeeding, similar results were obtained in the ELD and in S: streptozotocin opposes glycogen accumulation by increasing the lag and by lowering the accumulation rate (see e.g. Fig. 2). Interestingly, 40 mg/kg body wt was effective after refeeding, unlike the effect on ELD of fasted rats. The changes in glycogen after refeeding indicate that glycogen synthesis is impaired, which may be an appropriate explanation for the absolute decrease observed in the fasted animals.

Table 1. Effect of the i.v. injection of different doses of streptozotocin (see also Figs. 1 and 2) on blood glucose (expressed as g/l) of fasted rats and of rats given free access to food 2 hr after the injection

Hr after injection	0	2	4	6	8
Treatments			Plasma glucose levels		
1. Fasted rats					
Streptozotocin 40 mg/kg	0·89 ± 0·03 (5)	1·84 ± 0·25 (5)	1·74 ± 0·13 (5)	0·78 ± 0·07 (5)	0·38 ± 0·10 (5)
Streptozotocin 80 mg/kg	0·89 ± 0·07 (5)	2·14 ± 0·13 (5)	1·97 ± 0·23 (5)	0·30 ± 0·05 (5)	0·31 ± 0·07 (5)
2. Fed rats					
Streptozotocin 0 mg/kg	—	0·66 ± 0·07 (6)	1·68 ± 0·07 (6)	1·58 ± 0·06 (6)	1·56 ± 0·04 (6)
Streptozotocin 40 mg/kg	—	0·91 ± 0·14 (6)	3·65 ± 0·25 (6)	2·58 ± 0·61 (6)	2·52 ± 0·29 (6)
Streptozotocin 80 mg/kg	—	1·51 ± 0·03 (6)	5·32 ± 0·12 (6)	3·54 ± 0·42 (6)	3·30 ± 0·12 (6)

Results are expressed as mean ± S.E.M. with number of rats in parentheses.

The early increase in blood glucose after streptozotocin (see [1, 6]) is remarkably magnified by feeding the animals, which also prevents the subsequent hypoglycaemia (see [2]).

Table 2. Effect of the i.v. injection of different doses of streptozotocin (see also Figs. 1 and 2) on plasma insulin levels (expressed as mU/l) of fasted rats and of rats given free access to food 2 hr after the injection

Hr after the injection	0	2	4	6	8
Treatments			Plasma insulin levels		
1. Fasted rats					
Streptozotocin 40 mg/kg	12·1 ± 1·73 (5)	7·9 ± 0·73 (5)	4·1 ± 1·08 (5)	10·6 ± 3·02 (5)	23·8 ± 8·74 (5)
Streptozotocin 80 mg/kg	11·6 ± 1·53 (5)	13·0 ± 2·74 (5)	10·4 ± 5·89 (5)	43·4 ± 9·93 (5)	24·9 ± 3·24 (5)
2. Fed rats					
Streptozotocin 0 mg/kg	—	10·6 ± 1·11 (6)	27·8 ± 5·10 (6)	21·0 ± 3·58 (6)	29·3 ± 7·72 (6)
Streptozotocin 40 mg/kg	—	8·7 ± 0·87 (6)	15·3 ± 1·23 (6)	28·5 ± 9·86 (6)	36·2 ± 7·30 (6)
Streptozotocin 80 mg/kg	—	9·9 ± 0·89 (6)	15·6 ± 1·26 (6)	24·5 ± 3·52 (6)	32·6 ± 6·49 (6)

Results are given as mean ± S.E.M. with number of rats in parentheses.

The well known increase of plasma insulin after streptozotocin (see ref. 6)—which is apparent in the fasted rats after 6 hr ($P < 0.01$)—almost disappears in the fed rats. Most likely, this finding is at least partly related to the inhibition by streptozotocin of the postprandial increase of plasma insulin ($P < 0.01$).

If this is the case, the poor utilization of blood glucose to synthetize muscle glycogen may be an important clue to understanding the as yet unexplained simultaneous primary hyperglycaemia after streptozotocin (see Table 1). In fact, changes in the rate of gluconeogenesis do not seem important (primary hyperglycaemia is not dependent on the adrenal gland [5]) and liver glycogen increases (see Fig. 3 and Ref. 3).

In this connection, we should mention the possible role of the inhibition ($P < 0.01$) of the increase of IR-insulin after refeeding (Table 2), one of the earliest signs of impaired insulin secretion after streptozotocin so far reported in *in vivo* experiments. However, resistance to insulin might also be another possible factor: when comparing glycogen changes between 4 and 6 hr in the control, versus the period 6–8 hr in streptozotocin-treated rats, it becomes clear that the rate of glycogen accumulation at similar insulin levels is significantly lower ($P < 0.05$) in the treated rats, in spite of the higher blood glucose (see Fig. 2 and Tables 1 and 2).

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