The effect of tri-iodothyronine administration on protein synthesis in the diabetic rat

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(Received 25 April 1983/Accepted 7 June 1983)

In young male rats diabetes caused decreases in circulating free tri-iodothyronine and RNA concentration in liver and muscle, and in the rate of protein synthesis per unit of RNA (RNA activity) in muscle. Tri-iodothyronine treatment significantly increased RNA concentrations, but not RNA activity, in these tissues. Thus: (1) impaired thyroid status is a component of the diabetic condition; (2) tri-iodothyronine cannot stimulate the translational phase of protein synthesis in the diabetic rat.

We have previously reported that T_3 plays an important role in the regulation of protein synthesis in muscle by stimulating increases in RNA concentration and possibly the rate of translation (Brown *et al.*, 1981; Brown & Millward, 1983). Insulin also stimulates protein synthesis by increasing the rate of peptide-chain initiation (see Jefferson, 1980), and our previous results raised the possibility that T_3 might act on translation independently from insulin (Brown *et al.*, 1981; Brown & Millward, 1983). Given that a decrease in circulating thyroid hormone has often been reported in both human diabetes (Naeije *et al.*, 1976; Piroška & Foldes, 1976) and chemically induced animal diabetes (Jolin *et al.*, 1978; Dillmann, 1982) these results suggested that thyroid-hormone deficiency may participate in producing the decreased rates of protein synthesis characteristic of diabetes (Waterlow *et al.*, 1978). We report here an experiment performed to assess the effects of T_3 on protein synthesis in young diabetic rats *in vivo* in liver and in both oxidative and predominantly glycolytic muscles.

Materials and methods

Male Sprague–Dawley rats (Charles River U.K.), fed on Oxoid cubed diet *ad libitum*, were used. A total of 30 rats were divided into three groups at 65 g body wt., two of which were injected intravenously with 8 mg of streptozotocin (Sigma Chemical Co., Poole, Dorset, U.K.) in 0.3 ml of 0.05 M-sodium citrate buffer (pH 4.5), and the other was injected with buffer alone. Then 3 days later blood glucose was measured to confirm successful induction of

diabetes. At 5 days after injection, one group of the diabetic rats was implanted intraperitoneally with osmotic minipumps (Alzet model 2002; Alza Corp., Palo Alto, CA, U.S.A.) under diethyl ether anaesthesia. The pumps were primed with T_3 (Sigma Chemical Co.) dissolved in polyethylene glycol 300/water (3:1, v/v) at a concentration that would provide a total daily dose of 7.5 μ g of T_3 . All other rats underwent sham-implantation operations. Food intakes and body-weight changes were recorded daily, and after 7 days protein synthesis was measured by using the large-dose phenylalanine method (Garlick *et al.*, 1980). Rats were injected intravenously with 1 ml (per 100 g body wt.) of 150 mM unlabelled phenylalanine containing 50 μ Ci of L-[4- 3 H]phenylalanine (Amersham International, Amersham, Bucks., U.K.)/ml. At 2 and 10 min after injection, four and six rats were killed from each of the three groups respectively by decapitation, with collection of blood and rapid removal and cooling of liver and three skeletal muscles (gastrocnemius, plantaris and soleus). After weighing, tissues were frozen and stored in liquid N_2 .

The specific radioactivity (SR) of the free and protein-bound [3 H]phenylalanine in each tissue was determined by the method of Garlick *et al.* (1980).

The rate of protein synthesis (K_s) in each tissue was calculated from the SR of phenylalanine in protein (S_B) at 10 min, and the mean SR of free phenylalanine in the tissue between 0 and 10 min (S_A) from the formula:

$$K_s = \frac{S_B \times 100}{S_A \times t}$$

where t is the incorporation time in days and the units of K_s are %/day.

Abbreviation used: T_3 , tri-iodothyronine.

To obtain individual S_A values for the above calculation, the mean free SR values of the rats killed at 2 and 10 min within a group were extrapolated back to zero time, assuming a constant linear fall, and the arithmetic average of SR between 0 and 10 min ($\bar{S}_A$) was calculated. S_A values were then calculated from the formula:

$$S_A = S_{A(10)} \times \frac{\bar{S}_A}{\bar{S}_{A(10)}}$$

where $S_{A(10)}$ is an individual free SR from a rat killed at 10 min and $\bar{S}_{A(10)}$ is the group mean of the 10 min free-SR values.

The concentrations of protein and RNA in the tissues were determined as described previously (Brown *et al.*, 1981). RNA values were expressed as the RNA/protein ratio, indicating capacity for protein synthesis. RNA activity (fractional rate of protein synthesis divided by the RNA/protein ratio) was also calculated to indicate the rate of protein synthesis per unit of RNA (Millward *et al.*, 1976). Serum concentrations of total and free T_3 were measured by radioimmunoassay (Gruppo Lepetit liso-phase RIA; Uniscience Ltd., Cambridge, U.K.). Results are expressed as means $\pm$ s.d., and Student's *t* test was used to assess statistical significance.

Results

The growth rates of the sham-implanted groups indicated that pump-implantation surgery itself did not impair growth. However, the T_3 -treated diabetic group showed a transient growth impairment lasting 48 h after surgery, but growth then resumed at a rate

similar to that of the diabetic untreated group and indeed exceeded it during the final 48 h of the experiment. At pump implantation the mean weights were: controls 103 g, diabetics 78 g; mean weight gains over the 7-day treatment period were 60, 26 and 19 g in the control, diabetic and T_3 -treated diabetic groups respectively. Food intake per 100 g body wt. between days 2 and 7 was elevated by 70% in the diabetic rats and by 120% in the T_3 -treated diabetic rats compared with controls.

As shown in Table 1, the proportion of total weight accounted for by liver protein increased by over 50% in both diabetic groups compared with the controls ($P < 0.001$). In contrast, the proportion of body weight accounted for by the muscle protein in the diabetic untreated group decreased, by 34% and 39% ($P < 0.001$) in the gastrocnemius and plantaris muscles respectively, and by 8% (not statistically significant) in the soleus muscle. T_3 treatment exacerbated the decrease in all three muscles, with falls of 44% ($P < 0.001$), 55% ($P < 0.001$) and 35% ($P < 0.001$) in gastrocnemius, plantaris and soleus respectively compared with controls.

At the end of the experiment, serum total T_3 concentrations (ng/ml) were 1.03 ± 0.09 in the controls, 0.85 ± 0.30 in the diabetics and 4.85 ± 1.45 in the T_3 -treated diabetics. Free T_3 concentrations (pg/ml) were 8.6 ± 0.7 , 3.5 ± 1.3 and $35-45$ in the control, diabetic and T_3 -treated diabetic groups respectively. Although the decrease in total T_3 in the diabetic group was not significant at the 5% level ($P < 0.1$), the decrease in free T_3 was highly significant ($P < 0.001$).

As expected, diabetes markedly decreased protein synthesis in all four tissues under investigation ($P <$

Table 1. *Effect of T_3 treatment on protein synthesis and RNA content in liver and skeletal muscle in diabetic rats*
Rats were made diabetic with streptozotocin and treated with T_3 after 5 days by an implanted osmotic minipump providing $7.5 \mu\text{g}$ of T_3 /day for 7 days. After this time protein synthesis was measured *in vivo* by using a flooding dose of phenylalanine containing [^3H]phenylalanine as described in the text. Values are means $\pm$ s.d. for six rats.

	Liver	Gastrocnemius	Plantaris	Soleus
Protein content (mg/100 g body wt.)				
Control	713 ± 81	62.5 ± 4.7	11.9 ± 1.2	4.8 ± 0.6
Diabetic	1108 ± 124	41.0 ± 5.8	7.3 ± 1.2	4.4 ± 0.8
Diabetic + T_3	1097 ± 195	35.3 ± 6.0	5.4 ± 1.0	3.1 ± 0.5
Protein synthesis (%/day)				
Control	96.4 ± 2.7	15.5 ± 2.9	15.8 ± 1.6	17.3 ± 3.4
Diabetic	69.6 ± 16.9	6.8 ± 1.9	7.0 ± 2.3	9.1 ± 2.1
Diabetic + T_3	83.0 ± 16.3	7.7 ± 1.8	8.3 ± 2.8	10.6 ± 1.1
RNA/protein ratio (mg/g)				
Control	45.4 ± 5.4	8.6 ± 0.5	8.9 ± 0.7	14.3 ± 0.7
Diabetic	30.8 ± 3.3	5.2 ± 0.8	5.5 ± 0.6	10.8 ± 1.3
Diabetic + T_3	40.1 ± 5.2	7.0 ± 0.6	7.4 ± 0.9	14.7 ± 1.2
RNA activity (g of protein/day per g of RNA)				
Control	22.4 ± 2.4	18.1 ± 3.3	17.7 ± 1.8	14.5 ± 2.5
Diabetic	21.9 ± 3.8	12.0 ± 2.1	12.4 ± 2.6	8.1 ± 1.9
Diabetic + T_3	22.1 ± 7.7	11.1 ± 2.7	11.1 ± 3.7	7.2 ± 1.1

0.005 in all cases; see Table 1). T_3 treatment of diabetic rats increased the protein-synthesis rate in these tissues by 13–19%, but in no case was the increase statistically significant, and the values remained very much lower than those of control rats.

Table 1 also shows the tissue RNA content (expressed as RNA/protein ratio). Highly significant ($P < 0.001$) decreases in RNA content occurred in all four tissues of the diabetic rats. The greatest decreases were seen in the predominantly fast-twitch gastrocnemius and plantaris muscles (40% decreases in both cases), whereas in the liver and the predominantly slow-twitch soleus the decreases were 30% and 25% respectively. Treatment of diabetic rats with T_3 gave highly significant increases ($P < 0.001$) in RNA/protein ratios in all four tissues compared with the untreated diabetics. In the liver the value was still 10% below that of normal controls ($P < 0.05$). Also in the two fast-twitch muscles (gastrocnemius and plantaris) it was approx. 20% below normal ($P < 0.001$), i.e. values were intermediate between those for diabetics and controls, but significantly different from both. For the soleus muscle the increase brought RNA concentrations up to control values.

As far as changes in RNA activity are concerned, in the diabetic rats there were marked decreases in the three muscles, but in liver, unlike muscle, RNA activity was not depressed. T_3 treatment had no effect on RNA activity in any of the tissues studied.

Discussion

Previous work has indicated that the disposal rate of T_3 can be increased in diabetes (Brown, 1982) and that tissues can be less responsive to thyroid hormone in this condition (Dillmann, 1982). For this reason the dose of T_3 used in the present experiment was in excess of the physiological replacement, which is $<1 \mu\text{g}/\text{day}$ for young rats (100–150 g body wt.), although growth impairment does not occur in normal animals until doses of $>2 \mu\text{g}/\text{day}$ are used (Brown & Millward, 1983). As a result of the relatively high dose of T_3 used, the initial response of the diabetic rats given T_3 involved a severe check on an already decreased growth rate, which may have resulted from increased protein degradation, but this cannot be evaluated in these experiments. However, this was a temporary effect, and the improvement in growth rate on day 2 may reflect a more rapid degradation and disposal of T_3 .

The decreases in tissue protein-synthesis rates brought about by diabetes were consistent with previous reports in the literature (Pain & Garlick, 1974; Millward *et al.*, 1976; Jefferson, 1980; Odedra *et al.*, 1982), bearing in mind the duration of diabetes (12 days) and the method of measurement of protein

synthesis, which for the liver provides a total rate (i.e. including export proteins). In the present experiment the liver RNA activity remained unchanged, in contrast with the report by McNurlan & Garlick (1981), who, with a similar technique, reported 25% decreases in liver RNA activity in diabetes after 10 days duration. In their experiments liver protein-synthesis rate was decreased by 50%. Our results tend to emphasize the fact that the translational phase of protein synthesis in liver is not particularly sensitive to insulin.

Although the decrease in total serum T_3 in the diabetic group was only 18%, the free T_3 was decreased by 60%. Since it is the free fraction which is physiologically active, it is a better reflection of true thyroid status than the total T_3 concentration in the serum. The significance of this is that in previous experiments we have found that decreased circulating T_3 caused decreases in muscle RNA content [progressively more pronounced with decreasing concentration (Brown *et al.*, 1981; Brown & Millward, 1983)]. Thus the 60% decrease in circulating free T_3 may be an important contributory factor in the loss of RNA in the present experiments, especially since T_3 treatment partially reversed the change. Since the decrease in tissue RNA content in the diabetic rats was as great as, or greater than, that we have previously observed in thyroidectomized rats 10 days after surgery, the decreased free T_3 may not be completely responsible, however. An additional factor may be the increased concentrations of corticosterone in diabetes (Odedra *et al.*, 1982), since this hormone suppresses RNA content in muscle (Odedra & Millward, 1982) and eventually in liver (Odedra *et al.*, 1983). However, in the soleus muscle, diminished thyroid status may be the only factor involved in the decrease in RNA concentration, since T_3 treatment completely prevented this. This would be consistent with the observation that the soleus is less affected by steroid treatment than are fast-twitch muscles (Odedra & Millward, 1982), and it may well be that in soleus the whole effect of diabetes on RNA concentration is due to secondary thyroid-status impairment. Clearly in the fast-twitch muscles and in liver the hypothyroidism was only partially responsible for the decreased RNA concentrations.

Our previous experiments have indicated that T_3 is capable of stimulating the rate of protein synthesis in skeletal muscle through increases in RNA activity (Brown *et al.*, 1981; Brown & Millward, 1983). This was to some extent consistent with the reports that T_3 can increase the rate of peptide-chain elongation in other tissues (Mathews *et al.*, 1973; Carter *et al.*, 1975, 1976). Since we observed increased RNA activity despite depressed concentrations of circulating insulin, we interpreted this as indicating that T_3 might be capable of independent stimulation of

the translational phase of protein synthesis. The results of the present experiment do not confirm this possibility, since RNA activity was in no case increased from its low value in diabetic rats by T_3 treatment.

In conclusion, this experiment indicates that hypothyroidism does participate in the response to diabetes, being partly responsible for the decreases in tissue RNA content. However, in the absence of insulin exogenous T_3 is not able to increase depressed RNA activity. It may be that a minimal circulating insulin concentration is a prerequisite for any stimulation of RNA activity by T_3 . This requirement can be understood in the light of a proposal put forward in a previous paper (Brown *et al.*, 1981) that increases in RNA activity in skeletal muscle from low values in hypophysectomized or diabetic rats up to the lower end of the control range involve increases in initiation rates (which insulin is able to produce, but T_3 is not), whereas further increases in RNA-activity values reflect increased elongation rates, which could reflect the action of T_3 . Clearly this deserves further study.

These studies were generously supported by the Medical Research Council and the British Diabetic Association.

References

- Brown, J. G. (1982) Ph.D. Thesis, University of London
 Brown, J. G. & Millward, D. J. (1983) *Biochim. Biophys. Acta* **757**, 182–190
 Brown, J. G., Bates, P. C., Holliday, M. A. & Millward, D. J. (1981) *Biochem. J.* **194**, 771–782
 Carter, W. J., Faas, F. H. & Wynn, J. (1975) *J. Biol. Chem.* **250**, 3588–3594
 Carter, W. J., Faas, F. H. & Wynn, J. (1976) *Biochem. J.* **156**, 713–717
 Dillmann, W. H. (1982) *Metab. Clin. Exp.* **31**, 199–204
 Garlick, P. J., McNurlan, M. A. & Preedy, V. R. (1980) *Biochem. J.* **192**, 719–723
 Jefferson, L. S. (1980) *Diabetes* **29**, 487–495
 Jolin, T., Gonzalez, C. & Gonzalez, M. (1978) *Acta Endocrinol. (Copenhagen)* **88**, 506–516
 Mathews, R. W., Oronsky, A. & Haschemeyer, A. E. V. (1973) *J. Biol. Chem.* **248**, 1329–1333
 McNurlan, M. A. & Garlick, P. J. (1981) *Am. J. Physiol.* **241**, E238–E245
 Millward, D. J., Garlick, P. J., Nnanyelugo, D. O. & Waterlow, J. C. (1976) *Biochem. J.* **156**, 185–188
 Naeije, R., Clumeck, N., Somers, G., Vanhaelst, L. & Golstein, J. (1976) *Lancet* **i**, 1070–1071
 Odedra, B. R. & Millward, D. J. (1982) *Biochem. J.* **204**, 663–672
 Odedra, B. R., Dalal, S. S. & Millward, D. J. (1982) *Biochem. J.* **202**, 363–368
 Odedra, B. R., Bates, P. C. & Millward, D. J. (1983) *Biochem. J.* **214**, 617–627
 Pain, V. M. & Garlick, P. J. (1974) *J. Biol. Chem.* **249**, 4510–4514
 Pirooska, E. & Foldes, J. (1976) *Lancet* **i**, 1071
 Waterlow, J. C., Garlick, P. J. & Millward, D. J. (1978) *Protein Turnover in Mammalian Tissues and in the Whole Body*, pp. 667–671, Elsevier/North-Holland, Amsterdam