ACTION OF TRIIODOTHYRONINE ON THE SYNTHESIS OF RAT VENTRICULAR MYOSIN ISOENZYMES

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1. Introduction

Rat ventricular myosin has been shown to separate in pyrophosphate gels into 3 isoenzymes, V_1, V_2, V_3, in the order of decreasing electrophoretic mobility and ATPase activity [1]. These isoenzymes differ in heavy chain structure [2], their light chains not being different in molecular size or stoichiometry [1]. The distribution of these isoenzymes is a function of the thyroid state. V_3 predominates in rats rendered hypothyroid by hypophysectomy or thyroidectomy [1,2]. Replacement therapy with physiological doses of the hormone leads to a shift in distribution of isoenzymes towards V_1 [1]. Pure V_1 is present in adult animals treated with high doses of the hormone [2] or in untreated week 3–4 rats [1,2]. The change in myosin ATPase activity consequent upon a shift in isoenzyme distribution is presumably the biochemical basis for the modulation of cardiac contractility by thyroid hormone [1].

The mechanism of action of thyroid hormone on ventricular myosin isoenzymes has not been elucidated. Although the presence of nuclear binding sites for triiodothyronine (T_3) has been demonstrated in the heart [3], these may not be directly related to myosin since the hormone also regulates the Na^+-K^+-ATPase activity in this tissue [4]. The possibility exists that the myosin changes are secondary to changes in intracellular electrolytes [5] mediated by a change in Na^+-K^+-ATPase activity. Further, T_3 may act solely by exerting a differential effect on the rate of degradation of ventricular myosin isoenzymes rather than on their synthesis [1]. We report here studies on the regulation of ventricular myosin synthesis using a ventricular strip preparation. T_3 is shown to induce V_1 synthesis and suppress V_3 synthesis in the hypothyroid rat within hours of exposure to the hormone. The induced response can be demonstrated after in vitro exposure to T_3, and is abolished by actinomycin D pretreatment. These results suggest that thyroid hormone regulates myosin gene expression by a transcriptional mechanism.

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2. Experimental

2.1. *Treatment of animals*

Wistar rats were used. Rats were rendered hypothyroid by hypophysectomy [1]. Chronically hypophysectomized rats were injected with T_3 (2 mg/kg body wt i.p.). After 6,12,18,24 or 36 h, each animal was killed and a strip of the right ventricle was dissected for the analysis of myosin isoenzyme synthesis in vitro. To study the effect of actinomycin D on the action of T_3 on isoenzyme synthesis, hypophysectomized rats were pretreated with the drug (300–400 μg/kg i.p.) before the injection of T_3. Myosin synthesis was analysed 13–18 h after T_3 administration. The high mortality rate associated with more prolonged exposure of the hypophysectomized rat to actinomycin D rendered the analysis beyond this period difficult. To simplify analysis, data from actinomycin D treated rats which contained significant amounts of V_2 and V_1 isoenzymes were rejected.

2.2. *Analysis of myosin isoenzyme synthesis in rat ventricular strips in vitro*

A strip of the right ventricle measuring ~3 X 8 mm was tied to a glass rod and incubated in a test tube containing 2 ml of a medium maintained at 25°C in water bath. The incubation medium was a modified Krebs' solution containing 137 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM NaH_2PO_4, 24 mM $NaHCO_3$, glucose 45 mM, 250 μCi L-[4,5-^{3}H]-leucine (Amersham) and a mixture of amino acids (no leucine) at concentrations found in rat plasma [6,7]. The incubate was bubbled with a mixture of 95% O_2 and 5% CO_2. After incubation for 6 h, myosin was extracted and analysed in 4% polyacrylamide gels in pyrophosphate/glycerol buffer as in [1,8]. Gels were stained with Coomassie brilliant blue R. After scanning the gels in a densitometer (Gilford 240 with linear transport), gels were sliced ~1 mm intervals. The slices were treated with NCS tissue solubilizer (Amersham/Searle) and prepared for liquid scintillation counting according to [9]. Details of techniques used for the analysis of myosin synthesis will be elaborated elsewhere.

3. Results

3.1. *Synthesis of myosin isoenzymes in ventricular strips of control rats*

Ventricular myosin isoenzyme synthesis in the cardiac strip from a chronically hypophysectomized rat not treated with T_3 is shown in fig.1C. The protein profile shows a predominance of V_3 typical of hypophysectomized rats. The profile of radioactivity closely matches the profile of the protein, showing that the myosin isoenzymes synthesized is representative of those present before incubation. Essentially the same results were obtained in two other untreated hypophysectomized rats. These results are summarized in histograms shown in fig.3A. In another experiment a ventricular strip from a week 3 rat which contained only V_1 was used. A single peak of radioactivity coincident with V_1 was detected. These experiments establish that isoenzymes synthesized in vitro faithfully reflected those present in vivo.

3.2. *Synthesis of myosin isoenzymes in ventricular strips from T_3-treated hypophysectomized rats*

Figure 1 shows the effect, on the synthesis of ventricular myosin isoenzymes in cardiac strips from hypophysectomized rats, of a single injection of T_3, 12 h (fig.1B) and 36 h (fig.1A) before incubation in [^{3}H]leucine. Although the distribution of the 3

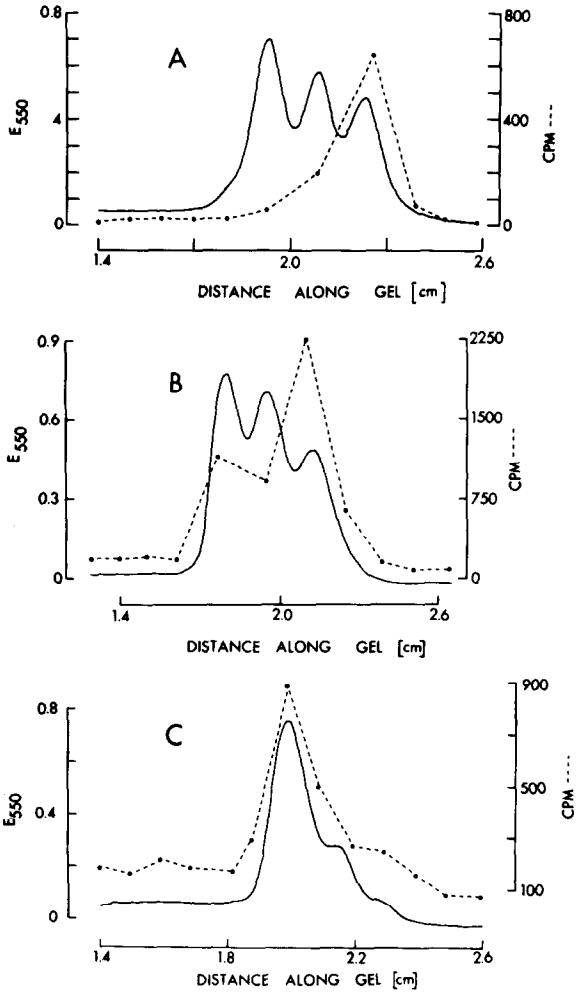

Fig.1. Action of T_3 on in vitro myosin isoenzyme synthesis in ventricular strips from hypophysectomized rats. The curves are protein (continuous) and radioactivity (broken) profiles of myosin after polyacrylamide gel electrophoresis [1]. In each case the protein peaks are, from right to left, V_1, V_2, V_3. Animals were injected with T_3 36 h (A) and 12 h (B) before excision of the heart for incubation in [^{3}H]leucine. (C) is from an uninjected hypophysectomized control.

isoenzymes in these and other hypophysectomized rats tended to vary, in every case T_3 treatment changed the distribution of radioactivity relative to the protein: the incorporation of label into V_3 was reduced while incorporation into V_1 was enhanced relative to the content of these isoenzymes. This change in the pattern of myosin synthesis was already well established after 12 h exposure to T_3 in vivo. By 36 h, very little V_3 was being synthesized. The time-course of this shift in isoenzyme synthesis derived from 10 expts with T_3 treatment is shown in fig.2. The radioactivity in V_1 and V_3, expressed as a % of total cpm, is plotted here against time after injection at which ventricular strip incubation commenced. The reciprocal nature of the changes in V_1 and V_3 synthesis over the 36 h period is similar to the changes in the content of these isoenzymes over 16 days in hypophysectomized rats given thyroid replacement therapy [1].

The radioactivity of V_1 in all T_3 treated preparations was the highest among the isoenzymes except at 6 h after treatment, for which V_2 contained 46% of the total myosin radioactivity but only 25% of the myosin. In this experiment V_1 contained 28% of the myosin radioactivity and 11% of the myosin while the corresponding figures for V_3 were 26% and 64%, respectively. Thus, the shift in isoenzyme synthesis from V_3 to V_1 is already evident after 6 h of in vivo exposure to T_3 followed by 6 h incubation in vitro. The data imply that the latent period for the action of T_3 on myosin synthesis, when the hormone is injected in vivo, is <12 h. It is noteworthy that only at this early stage of the action of T_3 does synthesis of V_2 come into prominence relative to the synthesis of V_1. The significance of this fact will emerge below.

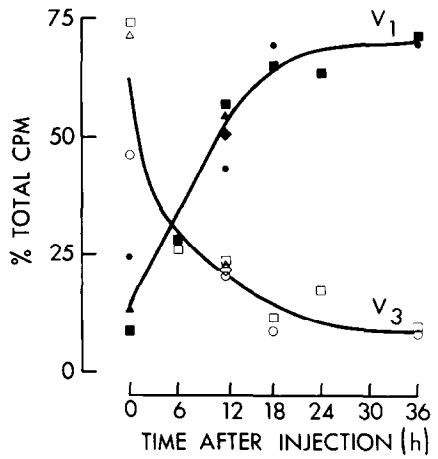

Fig.2. The time course of changes in ventricular myosin isoenzyme synthesis after a single injection of T_3. The radioactivity in V_1 (filled symbols), V_3 (open symbols), expressed as a percentage of total myosin in cpm, is plotted as time after T_3 injection. Each point is based on the mean % value obtained from 3–5 electrophoretic analyses of the same sample. Symbols of matching shape are for the 2 different isoenzymes in the same sample. Values at zero time are obtained from 3 untreated hypophysectomized rats.

3.3. *Effect of in vitro T_3 treatment on ventricular myosin isoenzyme synthesis*

Ventricular strips from 2 hypophysectomized rats were preincubated for 12 h in Krebs' solution in the presence of 4.5 μM T_3 at 25°C prior to the incubation in [^{3}H]leucine in the usual manner. Figure 3B

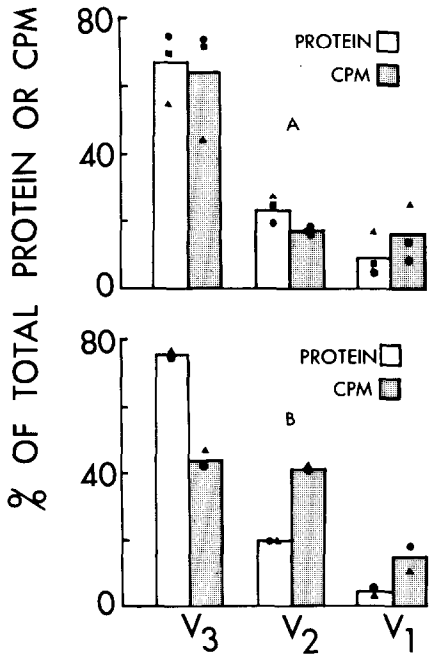

Fig.3. Effect of in vitro T_3 treatment on ventricular myosin isoenzyme synthesis. The histograms show the distribution of protein and radioactivity between the 3 ventricular myosin isoenzymes after incubation in [^{3}H]leucine for 6 h. (A) Untreated ventricular strips from 3 hypophysectomized rats. (B) Strips (from 2 hypophysectomized rats) preincubated for 12 h in the presence of 4.5 μM T_3. Matching symbols represent isoenzymes from the same sample.

shows the distribution of protein and radioactivity between the 3 ventricular myosin isoenzymes obtained from these experiments. Compared with data from hypophysectomized ventricular strips without T_3 treatment (fig.3A), an increase in V_2 synthesis and possibly some increase in V_1 synthesis at the expense of the synthesis of V_3 can be seen. These results are very similar to 6 h exposure to T_3 in vivo and are indicative of early response to the hormone. In another experiment, two ventricular strips were dissected from the same heart of an hypophysectomized rat. One strip was preincubated for 6 h with T_3 at 25°C, the other in a medium at 25°C without T_3. Both strips were transferred to the radioactive medium for the analysis of myosin synthesis. The pattern of isoenzyme synthesis in both strips was the same as in hypophysectomized controls described above. These experiments demonstrate that T_3 acts directly on the myocardium to bring about the shift in isoenzyme synthesis and that the latent period of this action in vitro at 25°C is 12–18 h.

3.4. *Effect of actinomycin D on the T_3-induced changes in ventricular myosin synthesis*

The effect of priming hypophysectomized rats with actinomycin D on the response to T_3 in ventricular myosin synthesis was investigated in 4 animals which contained virtually pure V_3. T_3 was injected 2 h after actinomycin D pretreatment. Analysis of myosin synthesis was initiated 13-18 h after T_3, i.e., well beyond the latent period for the T_3 response after in vivo exposure to the hormone. Figure 4A shows the result of one of these experiments in which incubation in [^{3}H]leucine was done 18 h after T_3 injection. Radioactivity is confined to V_3. This is more clearly shown in fig.4B in which myosin from this experiment had been mixed with non-radioactive, pure V_1 isoenzyme in order to locate V_1 in the gel. The absence of radioactivity in V_1 stands in sharp contrast with the result of the control (fig.4C) in which the animal was treated with T_3 alone for the same duration. Essentially identical results have been obtained for 3 other rats primed with actinomycin D and treated with T_3 for 13 h (2 rats) and 15 h. It is clear from fig.2 that marked shifts in isoenzyme synthesis should have already occurred after 12 h of exposure to T_3. Failure to synthesize V_1 implies a suppression of V_1 synthesis by actinomycin D. In none of these experiments was there any evidence for the enhancement of V_2 synthesis, an early sign of T_3-induced response. The persistence of V_3 synthesis is suggestive of the possibility that the suppression of V_3 synthesis may also be blocked by actinomycin D, but available data cannot unequivocally distinguish this from the

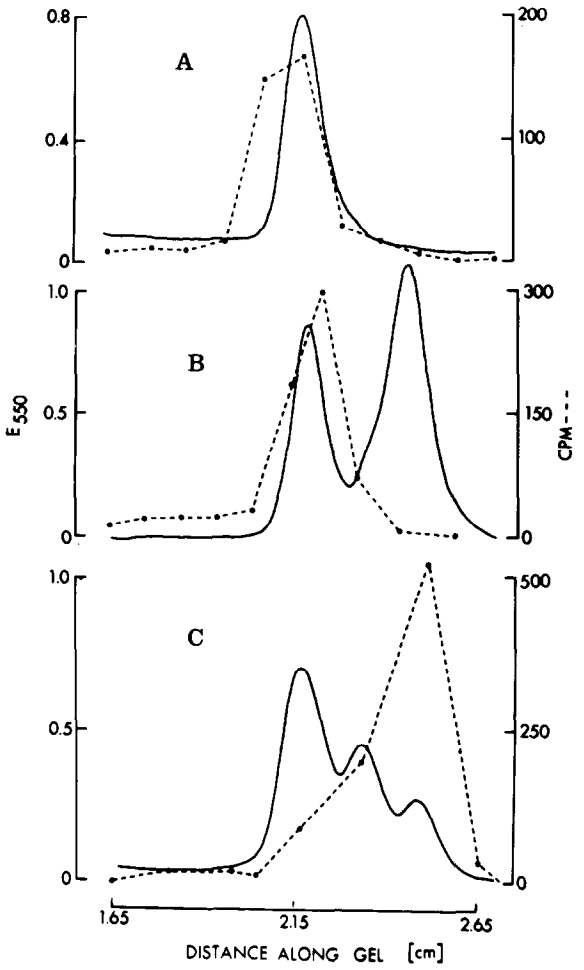

Fig.4. Effect of actinomycin D on the T_3-induced changes in ventricular myosin isoenzyme synthesis. Protein and radioactivity profiles of myosin isoenzymes are displayed as in fig.1. (A) Hypophysectomized rat pretreated with actinomycin D 2 h before T_3 injection, incubation in [^{3}H]leucine was done 18 h after T_3. (B) Same myosin sample as (A) coelectrophoresed with pure non-radioactive V_1 isoenzyme to indicate lack of incorporation of label into V_1. (C) Control with T_3 injection 18 h before incubation.

possibility that this merely reflects incomplete suppression of V_3 synthesis by T_3 within the period of T_3 treatment studied.

4. Discussion

4.1. *Nature of the response to T_3*

The experiments reported demonstrate that thyroid hormone in the hypothyroid rat induces V_1 synthesis and suppresses V_3 synthesis within hours of the exposure of the heart to the hormone. These actions per se may suffice to explain the shift in isoenzyme content of hypothyroid rats treated with thyroxine [1], though T_3 may also affect the rate of degradation of myosin isoenzymes differentially.

Since the light chains of ventricular myosin isoenzymes do not differ in molecular size or stoichiometry [1], it may be concluded that the action of the hormone is on heavy chain synthesis. As the heavy chain compositions of V_1, V_2 and V_3 are $(HC_\alpha)_2$, $HC_\alpha HC_\beta$ and $(HC_\beta)_2$, respectively, where HC_α and HC_β are two structurally distinct chains [2], it may be concluded that T_3 induces the synthesis of HC_α and suppresses the synthesis of HC_β. The early transient increase in incorporation of radioactivity into V_2, the heavy chain heterodimer, would simply reflect the initial stage of this reciprocal action of the hormone during which HC_α synthesis is beginning to increase while HC_β synthesis still predominates, a situation physicochemically favouring the formation of heterodimers over HC_α homodimers. The transient V_2 response further implies that the reciprocal control of the expression of ventricular myosin heavy chain genes by T_3 takes place within the same cell.

4.2. *Mechanism of action of T_3 on ventricular myosin gene expression*

The fact that similar changes in myosin isoenzyme synthesis can be brought about by in vitro exposure to T_3 shows that the hormone acts directly on the myocardium and not through its influence on other parts of the body. Since the latent period for the myosin response in vivo is <12 h, the response cannot be secondary to T_3-induced increase in Na^+-K^+-ATPase activity or the increased intracellular K^+ concentration as these changes are detectable only after 24–48 h [5]. It may thus be concluded that the observed changes in myosin isoenzyme synthesis are the result of T_3 itself acting directly on the cellular mechanisms for gene expression in the myocardium, independent of other known effects upon it.

The action of T_3 in inducing HC_α synthesis may be at the transcriptional or post-transcriptional level. The actinomycin D experiments suggest the former mechanism. Since these experiments were done on ventricular strips containing no V_1, any V_1 synthesis under T_3 stimulation would be de novo synthesis attributable to the administered hormone. The fact that no V_1 synthesis took place under T_3 stimulation after actinomycin D pretreatment suggests that induction of HC_α synthesis requires DNA-dependent RNA synthesis. It is unlikely that V_1 synthesis was merely delayed by the suppression of rRNA synthesis mediated by actinomycin D since:

(i) V_3 synthesis persisted at a significant level;
(ii) Enhancement of V_2 synthesis, the early sign of HC_α induction, was not observed.

It appears likely that failure to induce HC_α synthesis arose from the inhibition of HC_α mRNA synthesis. Further work, at the mRNA level, would be necessary to establish this. Nevertheless, the results reported here together with the modulating effect of T_3 on the level of hepatic mRNA for α_{2u} globulin [10] and of growth hormone mRNA in cultured pituitary cells [11] suggest that control of the transcription of genetic information may be the general mechanism of action of T_3 in its regulation of the synthesis of specific proteins.

The mechanism of the suppressive action of T_3 on HC_β synthesis is of considerable interest. T_3 is also known to suppress larval haemoglobin in the amphibian [12] and prolactin secretion in cultured pituitary cells [13], but the mechanism of suppression is poorly understood. From the data described above, it is uncertain whether the persistence of V_3 synthesis implies that HC_β suppressing effect of T_3 has been inhibited. Further experiments with more prolonged exposure to actinomycin D as well as mRNA analysis will be needed to clarify the mechanism of this suppressive action of T_3.

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