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EFFECT OF CHROMATIN ASSOCIATED FACTORS ON THE ACTIVITY OF THYROID HORMONE RECEPTORS IN RAT LIVER AND BRAIN.

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Summary
Receptors for thyroid hormones were extracted by 0.4 M KCl from the nuclei of rat liver and brain, and their binding properties compared to the properties of these receptors in unextracted nuclear suspensions. The inhibitory effect of a non-iodinated thyroid hormone analogue, 3,5,dimethyl-3'-isopropyl-l-thyronine (DIMIT) on [125I]-T3 binding was observed in the nuclear suspension of brain, but absent when the solubilized receptors of the same organ were tested. The initial properties of the receptor could be restored in a system containing the receptor and the extracted chromatin. Moreover, when the liver solubilized receptor was supplemented with the brain chromatin extract, the hepatic receptor acquired the binding ability of the brain receptors. The data suggest that chromatin associated components may confer organ specificity in thyroid hormone effects, and play a role in the selectivity of the recognition of thyroid hormone analogues by the receptor.

INTRODUCTION

Thyroid hormones bind to intranuclear chromatin-linked receptors; several lines of evidence indicate that this binding plays a significant role in eliciting thyroid hormone effects. Studies of the properties of these receptors show that they are acidic chromosomal proteins with estimated molecular weight of 50,000 to 70,000 (1-3). Changes in the number and in the affinity of the receptor for thyroid hormones have been described in various conditions : development (4-8), fasting (9-11) and hormonal status (12). Although the solubilized receptors prepared from different organs appeared to be identical as judged from chromatographic analysis, and from their behaviour versus various analogues of thyroid hormones (13,14), it has been suggested that the receptor activity and specificity could be regulated by protein extracted from the chromatin or by histones (15-17). In this paper we present evidence for differences in

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the binding properties of the solubilized receptors extracted from the chromatin, and of the receptor in the unextracted nuclei. The data suggest that chromatin associated factors may affect the recognition of the binding site by analogues, and that these factors may be involved in organ specificity of thyroid hormone binding.

Methods

Nuclei were prepared from adult rat cerebrum, heart, liver and kidney according to Torresani et al. (18). Briefly, the tissues were homogenized in SMT-buffer (sucrose 0.32 M, $MgCl_2$ 1 mM, Tris-HCl 20 mM pH 7.85). The nuclear pellet obtained after centrifugation was washed, resuspended in Tris-HCl buffer and layered over a 2.3 M sucrose cushion (7 ml) and centrifuged for 70 min at 25,000 rpm in a SW27 Beckman rotor at 4° C. After centrifugation, the supernatant was removed, and the nuclear pellet treated for 30 min at 0° C with 0.4 M KCl to extract the nuclear binding proteins. The extracted proteins were separated by centrifugation at 45,000 rpm for 30 min in the 50Ti rotor. The pellet was resuspended in SMT buffer using a glass-glass Dounce homogenizer.

The binding of T_3 to the nuclear receptor was determined by incubating 100 µl (60 µg) of the solubilized receptor with [^{125}I]-T_3 (specific activity 570 mCi/mg, final concentration 5 x 10^{-11} M) and increasing amounts of T_3 or analogue at concentrations ranging from 10^{-11} to 10^{-6} M. Binding of [^{125}I]-T_3 at this last concentration was considered as non-specific. Incubations were performed in polystyrene tubes in a final volume of 200 µl. The medium contained 20 mM Tris-HCl (pH 7.85), 0.4 M KCl, 1 mM $MgCl_2$, 2 mM EDTA and 5 mM dithiothreitol (TKEM-buffer). In the experiments where extracted chromatin was added back to the solubilized receptor SMT buffer was used. The incubation was for 150 min at 22° C. Free hormone was separated from bound hormone by the addition of Dowex 1x8 (Cl^-) in the incubation tubes (1 ml of a resin suspension made of 8 gr per 100 ml of either SMT or TKEM buffer). The tubes were spun at 3,000 x g for 5 min and the radioactivity of the supernatant assayed.

RESULTS

The inhibition of [^{125}I]-T_3 binding to its nuclear receptor by unlabelled T_3 and by 3,5-dimethyl-3'-isopropyl-l-thyronine (DIMIT) is depicted in Fig. 1. The concentration of DIMIT required to inhibit 50 % of [^{125}I]-T_3 binding is about 100-fold higher than that of T_3, showing that this analogue is only a weak competitor for the T_3 binding site, when performing the test with the 0.4 M KCl solubilized nuclear receptor. This finding is at variance with our earlier data indicating that DIMIT was an efficient inhibitor of T_3 binding in brain but not in the liver and kidney in studies using the nuclear suspension rather than the solubilized receptor (19). The specificity in recognition of this analogue can be restored in reconstitution experiments whereby the solubilized nuclear receptor is supplemented with the extracted chromatin (Fig. 2). Indeed, the addition of chromatin extract from brain nuclei clearly shifts to the left the [^{125}I]-T_3 binding curves generated by addition of DIMIT both

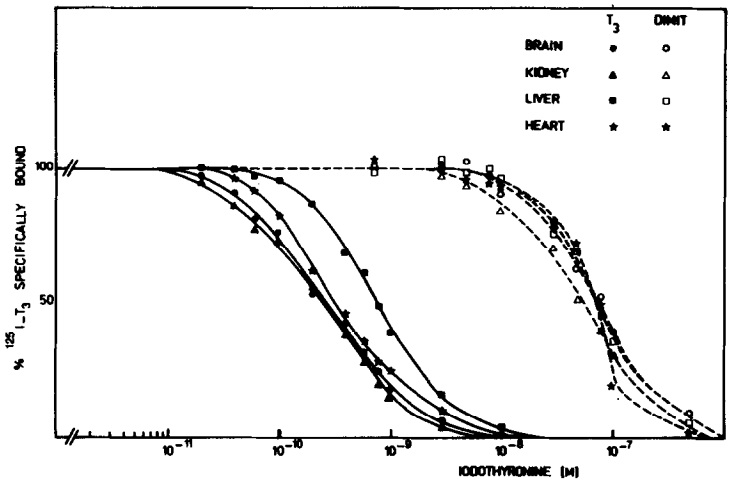

Fig. 1. Inhibition by unlabelled T_3 and DIMIT of [^{125}I]-T_3 binding to solubilized nuclear receptors from brain, kidney, liver and heart.

with liver and brain receptors, whereas the chromatin extract prepared from the liver nuclei is not effective. The chromatin itself does not exhibit T_3 binding ability (Fig. 3).

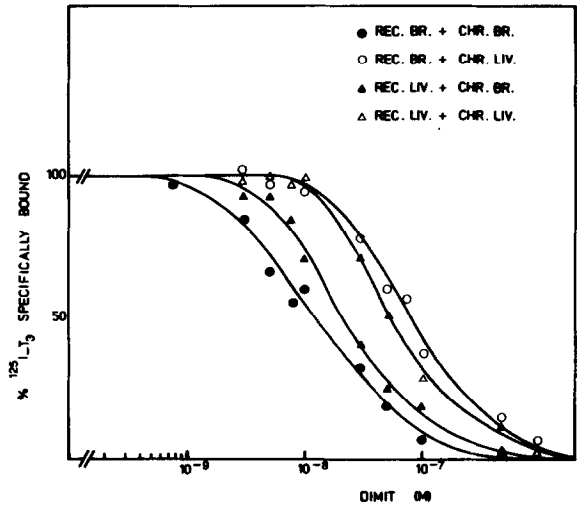

Fig. 2. Inhibition by DIMIT of [^{125}I]-T_3 binding to solubilized nuclear receptors from brain (o, ●) and liver (△,▲) in the presence of extracted chromatin prepared from brain (● , ▲) or from liver (o , △). In these reconstitution experiments the amount of extracted chromatin was added in the proportions existing in the original nuclear suspension.

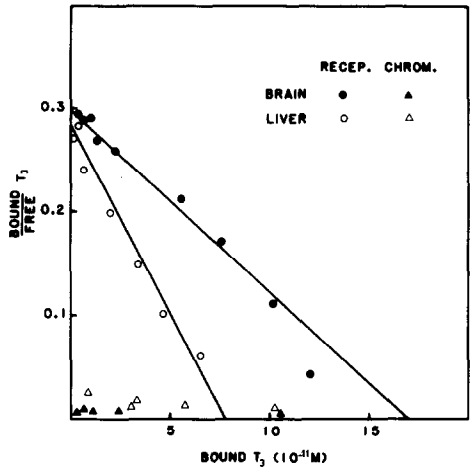

Fig. 3. Scatchard plot of the binding of T_3 to 0.4 M KCl solubilized receptors from brain (●) and liver (o). No significant binding of the hormone to the extracted chromatin from brain (▲) and from liver (△) is observed.

DISCUSSION

The data presented in this paper indicate that the receptor solubilized after salt extraction behaves differently from the receptor as studied in the nuclear suspension. Interestingly the binding properties present in the nuclear suspension can be restored to the solubilized receptor in a system using this receptor combined with the chromatin extract. Differences in the binding properties of the receptor during its attempted purification by column chromatography have been documented : by this procedure the receptor seems to lose its ability to bind T_3 but not T_4 (15). Other manipulations such as heat treatment and pH changes differently affect the number and possibly the affinity of T_3 and T_4 for the binding sites (16). Moreover, Eberhardt et al. (15) have shown that the high affinity T_3 binding capacity can be reconstituted by histone containing extracts of chromatin or purified core histones suggesting that the dissociation of the receptor from the chromatin alters the specificity of the receptor for thyroid hormones in a reversible way. These findings led Baxter et al. (1) to present a model of the thyroid hormone receptor -the holoreceptor- being made of a core receptor interacting with a regulatory subunit, possibly histones. In connection with a putative regulatory role of histones in the activity of the thyroid hormone receptor, Samuels et al. (17) have shown that the acetylation of chromatin bound proteins in GH1 cell nuclei results in a decrease of the number of nuclear associated thyroid hormone receptors

the acetylation lowering the affinity of the receptor for chromatin, without apparent modification in the ligand recognition by the receptor. From both kinds of data, it thus appears that the properties of the thyroid hormone receptor may be modified by histones or by other chromatin associated components. This interaction may therefore be considered as important in the gene expression controlled by T_3 or T_4 . Our findings suggest that the modulation of the binding ability of the receptor through chromatin linked factors may lead to organ specific effects of thyroid hormones in different target tissues. Moreover the observation that the inhibition of T_3 binding by DIMIT was also dependent on chromatin associated components indicates that in addition these components may act on the selectivity of the recognition of thyroid hormone analogues. An important implication of these results is that conclusions drawn from in vitro studies on the solubilized thyroid hormone receptors may be challenged due to the effects resulting from the binding of the receptor to the chromatin. Whether chromatin associated proteins also exert a regulatory role in the changes of the affinity for T_3 observed in brain nuclear receptors during development is currently under investigation (6).

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