Vol. 96, No. 3, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 16, 1980 Pages 1178-1183

SULFHYDRYL GROUPS REGULATE THYROID HORMONE BINDING AT NUCLEAR RECEPTOR SITES: FURTHER EVIDENCE FOR A SEPARATE BINDING SITE FOR REVERSE T_3

Howard C. Smith and Creswell J. Eastman

Endocrine Unit, Department of Medicine,

Westmead Centre, Westmead, N.S.W. 2145,

Australia

Received August 11,1980

SUMMARY

Pig and rat liver nuclei possess specific, high affinity, low capacity binding sites for 3,3',5'-triiodothyronine (reverse T_3) distinct from known 3,5,3'-triiodothyronine (T_3) binding sites. Sulfhydryl (SH) stabilising and oxidising agents have profound and opposite, but not equal, effects upon in vitro binding of reverse T_3 and T_3. In the absence of SH stabilising agents T_3 and reverse T_3 bind with similar affinity (Ka 0.83 x 10^9 v.s. 0.57 x 10^9 M^{-1}). SH stabilising agents produce a small increase in the binding affinity of T_3 and a profound decrease in the binding affinity of reverse T_3. Chromatography of nuclear protein preincubated with both radioligands revealed two separate peaks of protein bound radioactivity consistent with two nuclear binding sites. These data suggest that SH groups may regulate binding of T_3 and reverse T_3 to nuclear receptors, and provide a mechanism for biological action of reverse T_3.

INTRODUCTION

The initiation of thyroid hormone action is currently attributed to the interaction of 3,5,3' triiodothyronine (T_3) with specific nuclear binding sites (1, 2). These sites have been characterized as non-histone proteins which bind T_3 with high avidity and low capacity and bind analogues in accord with their known thyromimetic activity (3, 4). Additional evidence for the physiological importance of nuclear receptors for T_3 is the correlation between T_3 nuclear occupancy and synthesis of α glycerophosphate dehydrogenase, malic enzyme and growth hormone in appropriate thyroid hormone target tissues (1, 2).

There are, however, a number of physiological and pathophysiological states where maintenance of the euthyroid state is not readily explained by

0006-291X/80/191178-06$01.00/0

this mechanism. Such states are characterized by low serum concentrations of T_3, together with elevated serum concentrations of 3,3';5' triiodothyronine (reverse T_3), due to inhibition of extrathyroidal conversion of thyroxine (T_4) to T_3 (5). Using incubation conditions optimal for T_3 binding to its nuclear receptors, we have reported previously low affinity, high capacity reverse T_3 binding sites in hepatic nuclear protein (6). In this report we describe high affinity, low capacity nuclear binding sites specific for reverse T_3. Binding is dependent upon the redox state of sulfhydryl groups in the incubation mixture. Under certain physiological conditions, such sites may be of importance in the overall expression of thyroid hormone action.

METHODS

Whole pig or rat livers obtained within a few minutes of sacrifice were placed in liquid nitrogen. After crushing and thawing, tissue was homogenized in 3 volumes of buffer containing 0.32 M sucrose, 3 mM $MgCl_2$ and 10 mM Tris-Cl pH 7.85, using a Polytron homogenizer (Kinematica, Luzern).

Cell nuclei were prepared by the method of Samuels and Tsai (7) with more than 95% recovery of cell DNA. DNA was assayed by the method of Burton (8). Nuclear proteins were extracted in the above buffer mixture with 0.2 M $(NH_4)_2SO_4$, pH 7.85, by the method of Latham et al (9). This extract was used in binding studies without further purification.

10pM [^{125}I] labelled T_3 or reverse T_3 was incubated with nuclear protein and varying concentrations of unlabelled T_3 or reverse T_3 for 3 hours at 22° C. The incubation mixture contained 0.25 M sucrose, 5% glycerol, 2 mM EDTA, 0.1 M Na Cl and 20 mM Tris-Cl at pH 7.6 plus dithiothreitol (DTT), in the concentrations indicated in figures 1 and 2. At the end of incubation, protein bound hormone was separated from free hormone by passage of the incubation mix through small G25 medium Sephadex columns (8 x 0.5 cm) at 4°C.

Column chromatography (Sephadex G100, 28 x 1.5 cm) of nuclear protein preincubated with [^{125}I] T_3 or [^{125}I] reverse T_3 as previously described (6) was repeated using incubation and elution mixtures which did not contain thiol-active agents.

RESULTS

The effect of sulfhydryl group oxidising and stabilising agents on T_3 and reverse T_3 binding to the nuclear protein extract is shown in figures 1 and 2. Addition of the reducing agent dithiothreitol (DTT), to the incubation mix caused a graded increase in [^{125}I] T_3 bound from 9% (no DTT) to 14% (bound/total) in the presence of 5 mM DTT. Binding affinity calculated by Scatchard analysis was significantly increased in the presence of DTT, from Ka 0.83 x 10^9 to 3.8 x 10^9 ($P < 0.01$). Apparent binding capacity decreased from 0.16 ± 0.02

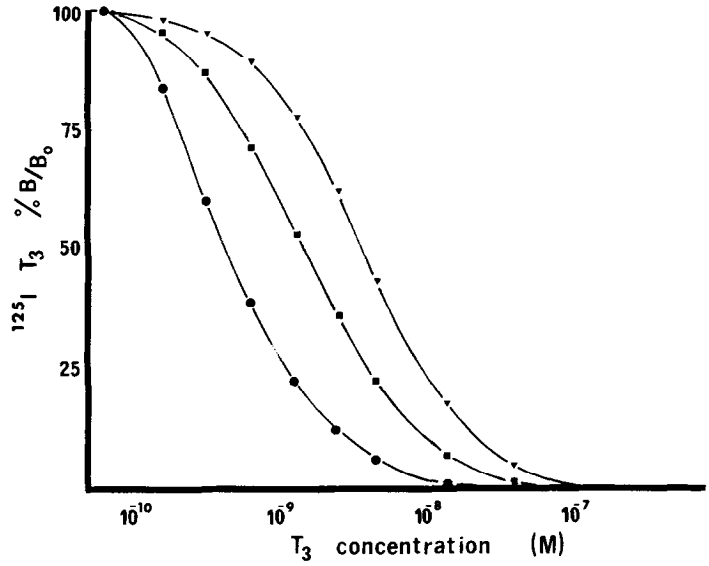

Fig. 1. Binding of [^{125}I] T_3 to hepatic nuclear protein in the presence of 5 mM DTT (●—●) or 5 mM GSSG (▼—▼) compared to its binding in the absence of thiol-active agents (■—■).

to 0.10 ± 0.07 pm/mg protein ($P < 0.02$). Similar results were obtained with rat and pig liver nuclear protein. The addition of 5 mM oxidised glutathione (GSSG) caused a decrease in [^{125}I] T_3 binding, in the absence of unlabelled T_3 from 9% to 4.5% (bound/total). Scatchard analysis showed a significant decrease in binding affinity from $0.83 \times 10^9 \pm 0.12$ M^{-1} to $0.48 \times 10^9 \pm$

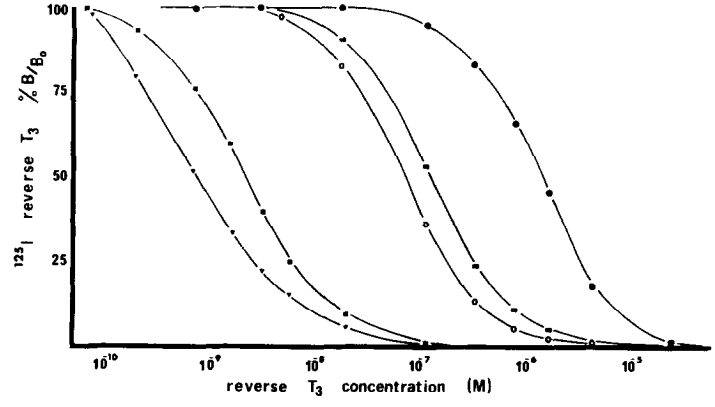

Fig. 2. Binding of [^{125}I] reverse T_3 to hepatic nuclear protein in the presence of 5 mM GSSG (▼—▼), 0.1 mM DTT (○—○), 5 mM mercaptoethanol (∞—∞) or 5 mM DTT (●—●), compared to its binding in the absence of thiol-active agents (■—■).

0.14 M^{-1} (n = 3: $P < 0.01$). Binding capacity decreased from 0.16 ± 0.02 to 0.11 ± 0.06 pm/mg protein (n = 3: $P > 0.05$). [^{125}I] T_3 binding was abolished by 10 mM GSSG and 5 mM iodacetamide.

By contrast, sulfhydryl group oxidising and stabilising agents had the opposite effect on [^{125}I] reverse T_3 binding to the nuclear protein extract. Binding affinity in the absence of added reagents was 0.57 x 10^9 ± 0.27 M^{-1} (n = 7), similar to that for T_3 binding. Addition of 5 mM DTT caused a marked decrease in the binding affinity to 0.33 x 10^7 ± 0.12 M^{-1} (n = 7: $P < 0.001$), and increased the binding capacity from 0.24 ± 0.06 to 233 ± 111 pm/mg protein (n = 7: $P < 0.001$). Small concentrations (0.1 mM) of DTT produced large changes in [^{125}I] reverse T_3 binding affinity and capacity. DTT and mercaptoethanol produced a dramatic increase from 16% to 42% in the binding of [^{125}I] reverse T_3 in the absence of unlabelled reverse T_3. Oxidised glutathione (5 mM) produced a small increase in binding affinity to 1.62 x 10^9 M^{-1}, and a decrease in binding capacity to 0.07 pm/mg protein (mean of duplicate experiments).

The elution profile of [^{125}I] T_3 bound to nuclear protein was compared with that of [^{125}I] reverse T_3 bound to nuclear protein. The bound [^{125}I] T_3 eluted with a peak in fraction 30. The major peak of [^{125}I] reverse T_3 bound to nuclear protein eluted with a peak in fraction 65. These peaks of protein bound, [^{125}I] labelled hormone were abolished by addition of 10^{-6} M non-radioactive T_3 or reverse T_3, to the incubation mixture. This elution profile was identical to that reported for the low affinity reverse T_3 binding site (6).

DISCUSSION

It has been assumed there is a single class of high affinity binding sites for thyroid hormone within the nucleus (1). Others have reported that reverse T_3 will displace [^{125}I] T_3 from these sites, albeit with low potency (9, 11). Inability to demonstrate high affinity binding of reverse T_3 was probably the result of routine inclusion of DTT or mercaptoethanol in

incubation mixtures. As our data show, thiol stabilising agents produce a profound decrease in reverse T_3 binding affinity with a concomitant increase in binding capacity.

The binding of T_3 and reverse T_3 changes in a reciprocal fashion dependent upon the status of sulfhydryl groups in the medium. The dramatic alterations in binding induced by sulfhydryl oxidising and reducing agents suggest a major regulatory role for sulfhydryl groups at the nuclear receptor site. Whether there are two separate binding proteins for T_3 and reverse T_3 or separate sites on the same protein undergoing conformational changes, remains to be determined. In addition, the mechanism whereby alterations in sulfhydryl groups alter iodothyronine binding remains unknown. As the putative T_3 receptor protein has been reported to contain SH groups (11), it is possible that the observed effects are due to conformational changes in the protein. In recent studies Eberhardt and co-workers have shown exposure of nuclear protein to heat or lowered pH induces major alterations in its binding characteristics (2, 12). They have proposed a model in which the thyroid hormone receptor can be converted to a form which retains binding activity for T_4, but which has reduced affinity for T_3. Our results could be interpreted in a similar manner, namely that there is a single binding site whose conformation is changed by the status of sulfhydryl groups. However, the ability to separate bound [^{125}I] T_3 from bound [^{125}I] reverse T_3, the additive binding of T_3 and reverse T_3 previously described (6) and the different rate of release of T_3 and reverse T_3 binding proteins from isolated whole nuclei (unpublished observations), are consistent with two distinct proteins. In the absence of a demonstrable biologic effect of reverse T_3 acting through a nuclear receptor, the physiologic significance of reverse T_3 binding is unknown.

Agents affecting sulfhydryl groups have profound effects on extrathyroidal monodeiodination of T_4 to T_3 and reverse T_3 (13, 14). The activity of the 5' (outer ring) deiodinase which deiodinates T_4 to T_3 is increased by

sulfhydryl reducing agents, and decreased by sulfhydryl oxidising agents. Thus, factors affecting iodothyronine production in vitro cause parallel changes in iodothyronine binding at the nuclear level. A recent study has provided evidence for substances in peripheral tissues which mimic the effects of DTT (15). In addition, the concentration of nonprotein sulfhydryl groups in the liver has been found to be significantly lower in fetal sheep than in adult sheep (14) and in the rat to be decreased by fasting (16). These results support the hypothesis that reverse T_3, acting through its own nuclear binding site, may play a significant role in the total expression of thyroid hormone action in the fetus and other states where serum T_3 is low, and serum reverse T_3 is high.

ACKNOWLEDGEMENTS

The valuable technical assistance given by Ms. Shirley Robinson, Ms. Kay Waite and Ms. Virginia Rashford and secretarial assistance given by Ms. Katharine Love is acknowledged. This work was supported by a grant from the National Health and Medical Research Council of Australia.

REFERENCES

1. Oppenheimer, J.H., (1979) Science, 203, 971-979.
2. Baxter, J.D., Eberhardt, N.L., Aprilette J.W., Johnson, L.K., Ivarie, R.D., Schacter, B.S., Morris, J.A., Seeburg, P.H., Goodman, H.M., Latham, K.R., Polansky, J.R., and Martial, J.A., (1979) Rec. Prog. Horm. Res., 35, 97-147.
3. Koerner, D., Schwartz, H.L., Surks, M.I., Oppenheimer, J.H., and Jorgensen, E.C., (1975) J. Biol. Chem. 250, 6417-6423.
4. Smith, H.C., Robinson, S.E., and Eastman, C.J., (1980) Endocrinology, 106, 1133-1136.
5. Cavalieri, R.R., and Rapoport, B., (1977) Ann. Rev. Med., 28, 57-65.
6. Smith, H.C., Robinson, S.E., and Eastman, C.J., (1980) Aust. J. Exp. Biol. Med. Sci. 58, 207-212.
7. Samuels, H.H., and Tsai, J.S., (1973) Proc. Natl. Acad. Sci. U.S.A., 70, 3488-3492.
8. Burton, K., (1956) Biochem. J., 62, 315-323.
9. Latham, K.R., Ring, J.C., and Baxter, J.D., (1976) J. Biol. Chem., 251, 7388-7397.
10. Papavasiliou, S.S., Martial, J.A., Latham, K.R., and Baxter, J.D., (1977) J. Clin. Invest., 60, 1230-1239.
11. De Groot, L.J., Refetoff, S., Strausser, J., and Barsano, C., (1974) Proc. Natl. Acad. Sci. U.S.A., 71, 4042-4046.
12. Eberhardt, N.L., Ring, J.C., Latham, K.R., and Baxter, J.D., (1979) J. Biol. Chem., 254, 8534-8539.
13. Visser, T.J., Van der Does-Trobe, I., Docter, R., and Hennemann, G., (1976) Biochem. J., 157, 479-482.
14. Chopra, I.J., (1978) Science, 199, 904-906.
15. Granberg, J.D., and Ballard, P.L., (1977) Endocrinology, 100, 1160-1168.
16. Tateishi, N., Higashi, T., Shinya, S., Naruse, A., and Sakamoto, Y., (1974) J. Biochem., 75, 93-103.