Vol. 80, No. 4, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 28, 1978 Pages 715-721

CHANGES IN THE SARCOLEMMA OF THE HYPOTHYROID HEART

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Received December 5,1977

SUMMARY: Sarcolemmal membranes prepared from the hearts of thyroidectomised sheep showed a fall of more than 90% in both 5'-nucleotidase activity and the number of β-adrenergic receptor sites. A fall of more than 60% in both Na^++K^+ATPase and adenyl cyclase activities also occurred. Either long-term or short-term treatment with thyroid hormones brought about concerted recovery of these pairs of sarcolemmal functions.

INTRODUCTION

Several of the physiological changes that occur in hypothyroidism such as the depressed basal metabolic rate and oxygen consumption of tissues (1), the lowered cardiac output (2) and myocardial contractility (3), the impaired performance of skeletal muscle (4,5) and the decreased rate of lipolysis (6) may be interpreted in terms of certain changes in the plasma membranes of thyroid-sensitive cells. Two lines of thought have emerged. The first of these relates some of the effects to a depressed responsiveness of hypothyroid tissues to adrenergic stimulation (7). This could occur either through a reduction in the number of β-adrenergic receptors on the cell surface or through an impairment of the activity of the catecholamine-responsive adenyl cyclase in the cell membrane and some evidence has been adduced for each of these effects (6, 8, 9). The second line of reasoning attributes the calorigenic effect of thyroid hormone to an effect on the activity of the Na^++K^+ATPase in the cell

0006-291X/78/0804-0715$01.00/0

membrane. Acting as a sodium ion pump this enzyme helps to maintain intracellular Na^+ and K^+ concentrations at levels appropriate to the cell's function. Its activity in a number of tissues has been shown to depend upon the thyroid status of the animal (10-12) and to contribute substantially to energy consumption in the cell.

The changes in heart function that occur when the thyroid status is changed show features consistent with both of these points of view. Under conditions of hyperthyroidism the heart shows an increased sensitivity to β-adrenergic agonists (7) and Williams et al. (8) and Ciaraldi and Marinetti (13) have recently shown an increased number of β-adrenergic receptor sites in particulate fractions prepared from the hearts of rats following treatment with relatively large doses of thyroid hormone. On the other hand Philipson and Edelman (14, 15) have shown that treatment of hypothyroid rats with thyroid hormone not only increases the activity of Na^++K^+ATPase in heart membrane preparations but also increases the intracellular K^+ concentration in heart muscle.

We have used thyroidectomised sheep to study the effects of thyroid hormone status on these functions and activities in sarcolemmal membranes prepared from ventricular heart muscle. In addition measurements were made of the activity of 5'-nucleotidase as a marker for sarcolemmal purity.

MATERIALS AND METHODS

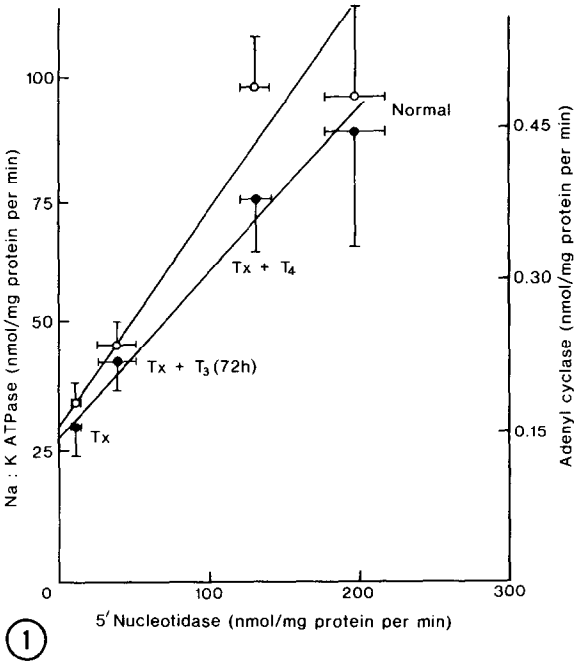
Fifteen Australian Merino wethers (body weight 24.2 ± 0.4 Kg) were surgically thyroidectomised at 8 months of age and maintained in roofed individual pens until killed 5-6 months later. Eight weeks after successful thyroidectomy (as determined by plasma thyroxine) five animals were selected at random and treated with L-thyroxine (0.5 mg suspended in 0.5 ml peanut oil injected subcutaneously) every second day until they were killed. A further control group of 5 normal wethers of the same age was maintained under similar conditions. All animals were fed ad lib. a diet of wheaten hay chaff and dried lucerne chaff (3:1 by weight). Three days prior to killing five of the untreated thyroidectomised

animals received a single intravenous injection of 0.5 mg L-triiodothyronine freshly dissolved, and administered in phosphate-buffered saline. At the time of killing plasma thyroxine levels in both untreated thyroidectomised animals and those that had been treated 72h earlier were <0.2 µg/100 ml. In the animals treated continuously with L-thyroxine, plasma thyroxine (mean ± SEM) was 11.2 ± 1.4 µg/100 ml, and in the normal animals it was 8.1 ± 0.5 µg/100 ml.

Animals were killed by captive-bolt pistol, a sample of ventricular heart muscle quickly removed, minced and chilled and sarcolemmal membranes prepared by the method of Kidwai (16). Specific binding of (-)-^{3}H-dihydroalprenolol to β-receptors was measured in duplicate samples of about 100 µg of sarcolemmal protein as described by Ciaraldi and Marinetti (13) except that the concentration of (-)-^{3}H-dihydroalprenolol was 20nM. 5'-Nucleotidase activity was measured by the method of Aronson and Touster (17). Duplicate tubes containing about 25 µg sarcolemmal protein were incubated for 30 min. and 60 min. and release of Pi measured. Na^++K^+ATPase was measured at pH 7.4 in volumes of 1.0 ml containing NaCl (100mM), KCl (20mM), $MgCl_2$ (3mM), EDTA (1mM), tris-ATP (2.5mM), tris-chloride buffer (50mM) and about 20 µg of sarcolemmal protein. Duplicate tubes were incubated at 37°C for 30 min. and 60 min. with and without 10^{-4}M ouabain. Reactions were stopped with 2.0 ml cold trichloroacetic acid (7.5% w/v) and Pi measured by the method of Fiske and Subbarow (18). Adenyl cyclase was estimated in duplicate at 30°C and pH 7.5 in final volumes of 0.2 ml containing tris-chloride buffer (50mM), $MgCl_2$ (5mM), theophylline (10mM), NaF (5mM) ATP (2mM, in the presence of creatine kinase and excess creatine phosphate) and about 60 µg of sarcolemmal protein. Reactions were started by adding ATP (together with its regenerating system) in a volume of 0.06 ml following 30 min. pre-incubation of the tubes at 30°C. Ten minutes after adding ATP the reaction was stopped by heating tubes for 3 min. in a boiling water bath. The supernatant was chilled and stored at -20°C prior to estimating cAMP by means of kits obtained from the Radiochemical Centre, Amersham, U.K. Protein was estimated by the method of Lowry et al. (19). Measurement of all sarcolemmal activities was carried out on the day of killing. Plasma thyroxine was measured by radioimmuno assay using kits from the Radiochemical Centre, Amersham, U.K.

RESULTS AND DISCUSSION

The results are set out graphically in Figs. 1 and 2 where group means (and SEM) for the three activities measured have been plotted as ordinates against the corresponding mean activities (and SEM) of 5'-nucleotidase; all results have been expressed on a protein basis. Fig. 1 shows an unexpected but very close linear correlation between the specific β-binding capacity of the sarcolemmal membranes and their 5'-nucleotidase activity. Reference to the group treatments indicated in Fig. 1



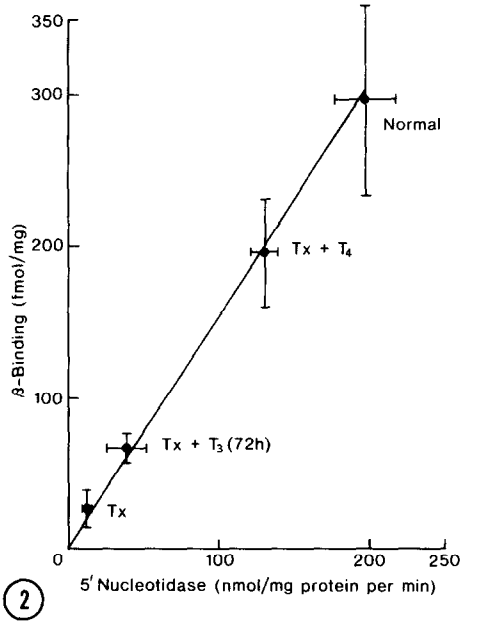


FIGURE 1. Specific binding of (-)-^{3}H-dihydroalprenolol to indicate the number of β-adrenergic receptor sites on sarcolemma from sheep heart plotted against 5'-nucleotidase activity of the same preparations. Treatments of the various groups are indicated on the figure near the appropriate values which show mean ±SEM for the two activities for the group concerned. Tx = untreated thyroidectomised animals; Tx + T3 (72 h) = thyroidectomised animals treated 72 h previously with 0.5 mg L-tri-iodothyronine; Tx + T4 = thyroidectomised animals treated continuously for 3-4 months with the equivalent of 250 µg of L-thyroxine per day; NORMAL = normal controls. Replication was 5.

FIGURE 2. Activities of $Na^{+}+K^{+}$ATPase (open circles) and adenyl cyclase (closed circles) plotted against 5'-nucleotidase activities in sheep heart sarcolemma. Treatments and replication as in Figure 1.

shows that both of these activities were very severely depleted (by more than 90%) by thyroidectomy and that both activities were substantially and equally restored by treatment with physiological doses of L-thyroxine. A smaller but again equal response of both activities was evident in animals treated 72 hours earlier with a single dose of tri-iodothyronine.

These results might be interpreted to mean that the

sarcolemmal preparations from hypothyroid animals contained much larger quantities (sufficient to cause a further ten-fold dilution of true sarcolemmal protein) of non-sarcolemmal protein than did preparations from the normal hearts. It could then be argued that the hypothyroid sarcolemma contained normal activity of 5'-nucleotidase and normal numbers of specific β-binding sites. Previous demonstrations of effects of thyroid hormone treatment on the specific β-binding capacity of particulate fractions from rat heart might then be supposed to have suffered from the same effect since marker enzymes were not measured (8, 13). There is, however, good reason to believe that the fall in activity of both parameters shown in Fig. 1 is real.

In Fig. 2 Na^++K^+ATPase and adenyl cyclase activities are seen to fall together when plotted (on appropriate scales) against 5'-nucleotidase activity, but they do not pass through the origin. If we are to accept the proposition that the concerted fall in 5'-nucleotidase activity and specific β-binding is attributable to dilution of sarcolemmal protein with non-sarcolemmal protein, then it follows from Fig. 2 that the hypothyroid sarcolemma shows an approximately five-fold increase in the activities of both Na^++K^+ATPase and adenyl cyclase as compared with the sarcolemma from normal heart. There is now a substantial body of evidence that Na^++K^+ATPase activity falls in several tissues including the heart of hypothyroid animals (10-12, 14, 15) and there is some evidence that adenyl cyclase activity, at least in some tissues, is also dependent for its activity upon thyroid hormone (6, 7, 9). The preparations of sarcolemma from our hypothyroid hearts showed no indication of the gross contamination that would seem to be necessary to have caused a ten-fold dilution of true sarcolemmal protein. The material is recovered as a

discrete and visible band from the topmost portion of a sucrose gradient and it is subsequently freed from any contaminating soluble protein. Recoveries of total protein in this fraction for the various treatments were as follows: normal, 1.5 ± 0.1 mg; Tx + T4, 1.4 ± 0.1 mg; Tx + T3 (72 h), 1.7 ± 0.2 mg; Tx, 1.9 ± 0.3 mg. These values do not suggest gross contamination.

The conclusion therefore seems justified that both specific β-binding and 5'-nucleotidase activity fell very sharply in hypothyroid sarcolemma and that both activities responded in concert to treatment with thyroid hormone. The results also indicate a more modest although still substantial fall in the activities of both $Na^+ + K^+$ATPase and adenyl cyclase. The cohesive way in which these pairs of sarcolemmal activities changed with changing thyroid status is of considerable interest. It may be significant that whereas both $Na^+ + K^+$ATPase and adenyl cyclase are plasma-membrane enzymes whose active centres are directed inward toward the cytoplasm, the active site of 5'-nucleotidase (20) and the binding sites for β-adrenergic agonists are both believed to reside on the outside surface of the plasma membrane.

ACKNOWLEDGEMENTS

We thank Dr. G.H. McIntosh for carrying out the surgical thyroidectomies and Miss D.M. Martin for assays of plasma thyroxine.

REFERENCES

1. Barker, S.B. and Klitgaard, H.M. (1952) Am. J. Physiol. 170, 81-86.

2. Strauer, B.E. and Schulze, W. (1976) Basic Res. Cardiol. 71, 624-644.

3. Buccino, R.A., Spann, J.F., Pool, P.E., Sonnenblick, E.H. and Braunwald, E. (1967) J. Clin. Invest. 46, 1669-1682.

4. Lambert, E.H., Underdahl, L.O., Beckett, S. and Mederos, L.O. (1951) J. Clin. Endocrinol. 11, 1186-1205.

5. Gold, H.K., Spann, J.F. Jr. and Braunwald, E. (1970) J. Clin. Invest. 49, 849-854.

6. Krishna, G., Hynie, S. and Brodie, B.B. (1968) Proc. Nat. Acad. Sci. 59, 884-889.

7. Guttler, R.B., Otis, C.L., Shaw, J.W., Warren, D.W. and Nicoloff, J.T. (1975) in Thyroid Hormone Metabolism, Ed. by Harland, W.A. and Orr, J.S. pp 201-211 Academic Press: London.

8. Williams, L.T., Lefkowitz, R.J., Watanabe, A.M., Hathaway, D.R. and Besch, H.R. Jr. (1977) J. Biol. Chem. 252, 2787-2789.

9. Will-Shahab, L., Wollenberger, A. and Küttner, I. (1976) Acta biol. med. germ. 35, 829-835.

10. Ismail-Beigi, F. and Edelman, I.S. (1970) Proc. Nat. Acad. Sci, USA 67, 1071-1078.

11. Ismail-Beigi, F. and Edelman, I.S. (1971) J. Gen. Physiol. 57, 710-722.

12. Lo, C-S. and Edelman, I.S. (1976) J. Biol. Chem. 251, 7834-7840.

13. Ciaraldi, T. and Marinetti, G.V. (1977) Biochem. Biophys. Res. Commun. 74, 984-991.

14. Philipson, K.D. and Edelman, I.S. (1977) Am. J. Physiol. 232, C196-C201.

15. Philipson, K.D. and Edelman, I.S. (1977) Am. J. Physiol. 232, C202-C206.

16. Kidwai, A.M. (1974) Methods in Enzymology 31A, 134-142.

17. Aronson, N.N. and Touster, O. (1974) Methods in Enzymology 31A, 90-102.

18. Fiske, C.H. and Subbarow, Y. (1925) J. Biol. Chem. 66, 375-400.

19. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.

20. Frick, G.P. and Lowenstein, J.M. (1976) J. Biol. Chem. 251, 6372-6378.