ALTERED RATIOS AND DIFFERENT SUBCELLULAR DISTRIBUTION OF TYPE I AND TYPE II cAMP-DEPENDENT PROTEIN KINASES IN SEVERAL THYROIDAL PATHOLOGICAL TISSUES

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1. Introduction

Thyrotropic hormone (TSH) controls thyroid hormone synthesis and secretion through the activation of the adenylate cyclase and the increase of the intracellular level of cAMP (see references in [1]). The cAMP formed, activates the cAMP-dependent protein kinases [2–5]. If TSH stimulation is prolonged, modifications were also observed in the ratios between the two protein kinase isoenzymes present in thyroid cytosol (type I and type II). It was thus demonstrated that in chronically stimulated rat thyroid glands the activity of type II kinase is selectively increased [6–8]. The modifications of ratios between type I and type II protein kinases were also noted during the cell cycle of cultured Chinese hamster ovary cells [9–11], during the differentiation and postnatal development of several organs [12–15], after stimulation of different tissues by trophic hormones [16–23] as well as in some tumoral tissues [24] and transformed cells [25]. All these findings suggest that the two isoenzymes of cAMP-dependent protein kinases could have different biological functions, and that their activity may be controled by separate and distinct mechanisms, as was demonstrated for enzymes from rabbit uterus [26].

Here, we were interested to see whether and how the cAMP-dependent protein kinases are modified in human thyroids expressing different troubles of hormonogenesis. Our results showed that, with respect to normal glands, the alterations of the ratios between the two cAMP-dependent protein kinase isoenzymes, and their different subcellular distributions occured in several types of pathological tissues. The observed quantitative changes were due to the selective modifications of the type I protein kinase activity: in toxic adenoma this activity was significantly inhibited, while in non-toxic adenoma it was stimulated, with respect to control tissues. These data suggest that cAMP-dependent protein kinases could be involved in the mechanism of different thyroidal troubles in a very specific and selective way.

2. Material and methods

Immediately after excision, glands were frozen and stored at −80°C until used. Thawed glands were cleaned from fibrous and other adjacent tissues, and homogenized in 15 vol. (w/v) 50 mM Tris–HCl buffer (pH 7.4) containing: 25 mM KCl, 5mM $MgCl_2$, 3 mM mercaptoethanol and 0.25 M sucrose, under the same conditions as for rat glands [6,27]. After separation of cytosol, and prior to analysis, the particulate enzymes were solubilized with Nonidet P 40: 0.5% final conc. (w/v) in the homogenization buffer, for 1 h at 4°C, followed by dilution of Nonidet to 0.3% and centrifugation for 1 h at 50 000 rev./min, Rotor 50. This solubilized the totality of particulate cAMP-dependent protein kinases.

Here, we analyzed the protein kinase activity in 2 pieces from normal glands, 3 pieces from colloid nodular goiters, 2 toxic adenomas and 1 non-toxic adenoma. Toxic adenomas were hot nodules with increased iodine uptake and metabolism. The non-toxic adenoma was a hot nodule with increased uptake of iodine, which was, however, almost completely discharged by the perchlorate, indicating that iodine, taken up by the nodule, was not further metabolized.

All nodules were identified by clinical and histological criteria. They were always surrounded by a thick fibrous capsule.

All other techniques (cytosol fractionation and protein kinase determination) have been described in [6,27]. Results are expressed either in % of total activity determined for each type of tissue (pmol ^{32}P incorporated into histone/mg tissue in 5 min incubation), or relatively, with respect to control tissue (normal glands).

3. Results and discussion

In thyroid gland cytosols the two cAMP-dependent protein kinases (types I,II) have been identified by several authors [7,27–30]. Their relative activities depended on the origin of tissue (species) and the hormonal status of the glands [6–8].

In pathological tissues, analyzed here, 3 main modifications of cAMP-dependent protein kinases were observed:

(i) Ratios between type I and type II protein kinases were significantly different, depending on the origin of tissue: in normal tissue and in non-toxic adenoma type I kinase was predominant; in toxic adenomas the two kinases were found in the same proportions; while in colloid nodular goiters kinase II was preponderant (fig.1).

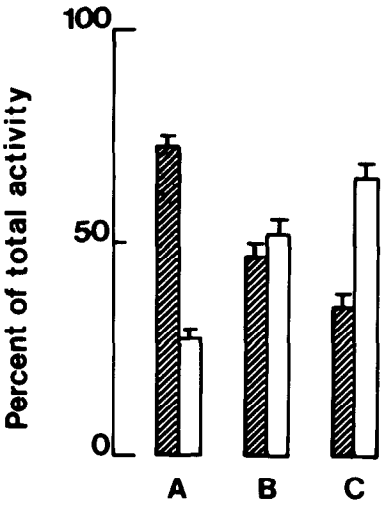

Fig.1. Relative activities of type I (▨) and type II (□) protein kinases in: (A) normal tissues; (B) toxic adenomas; (C) colloid nodular goiters.

Table 1
Particulate protein kinase activity

Tissue	Percent of total activity
Normal	7.5 ± 0.8
Non-toxic adenoma	8.4 ± 1.0
Toxic adenoma	17.9 ± 3.2[a]
Colloid nodular goiter	16.2 ± 1.4[b]

[a] $P < 0.02$; [b] $P < 0.001$

(ii) Qualitative and quantitative differences in the subcellular distribution of the two types of kinases were noted. In both, colloid nodular goiters and toxic adenomas the proportion of particulate enzymes was increased significantly (table 1). Moreover, while in normal glands and in non-toxic adenoma, type I kinase was found almost exclusively; in colloid nodular goiters and in toxic adenomas 2 peaks of protein kinase activity were present in the particulate fraction (fig.2).

(iii) The estimation of quantitative difference between normal and pathological tissues was possible only in 2 cases in which both tissues were obtained from the same patient: in the case of non-toxic

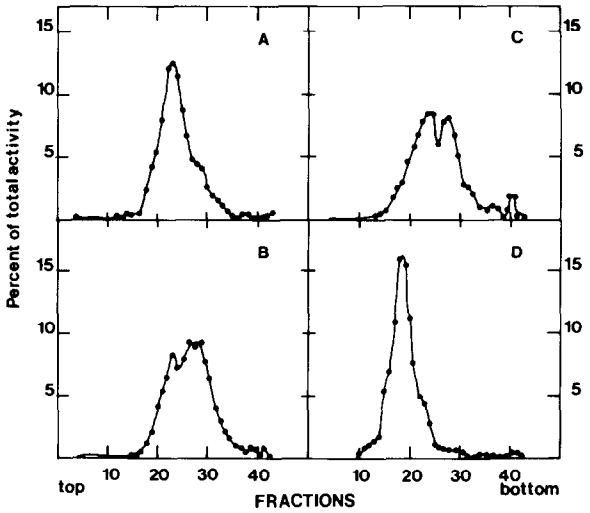

Fig.2. Sucrose gradient ultracentrifugation patterns of particulate protein kinases solubilized from: (A) normal tissue; (B) colloid nodular goiter; (C) toxic adenoma; (D) non-toxic adenoma. Results are expressed in % of total particulate activity.

Table 2
Quantitative evaluation of protein kinase activity (pmol ^{32}P . mg tissue^{-1} . 5 min^{-1}) in non-toxic and toxic adenomas

	Total[a]	Type I	Type II
Control	21.3	15.9	5.4
Non-toxic adenoma	32.2	26.5	5.7
Percent of control	151	167	106
Control	48.8	33.8	15.0
Toxic adenoma	28.5	12.6	15.9
Percent of control	58.4	37.3	106

[a] Cytosol + particulate activity

adenoma and in 1 of the 2 cases of toxic adenomas. With respect to normal tissue, in non-toxic adenoma the total protein kinase activity was stimulated, while in toxic adenoma it was inhibited. In both cases the modifications were due only to the selective stimulation or inhibition of type I kinase (table 2).

Since the total activity of type II kinase was not increased in toxic adenoma, the appearance of this activity in the particulate fraction represents probably the translocation of a part of the cytosolic enzyme (table 3).

In addition it is important to point out that in the case of toxic adenoma, the tissue which surrounded the encapsulated nodule (perinodular region) had protein kinase patterns which were intermediate between those obtained in normal and in nodular part of the gland (table 3). This indicated that pathological changes were not limited only to the encapsulated nodule, but were propagated also to the extracapsular tissue.

We still do no know either the exact role of the two kinases, or the mechanism by which they control thyroid hormone synthesis and secretion, the important changes of these enzyme activities observed in different pathological tissues suggest that some thyroidal troubles could be due not only to modifications of enzymes directly involved in the hormonogenesis, like peroxidase [31–33], but also to the alterations of the regulatory enzymes, like protein kinases, which control the phosphorylation of different thyroid cell proteins.

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References

[1] Dumont, J. E. (1971) Vitam. Horm. 29, 287–412.
[2] Spaulding, S. W. and Burrow, G. N. (1974) Biochem. Biophys. Res. Commun. 59, 386–391.
[3] Spaulding, S. W. and Burrow, G. N. (1975) Endocrinology 96, 1018–1021.
[4] Schumacher, M. and Hilz, H. (1978) Biochem. Biophys. Res. Commun. 80, 511–518.
[5] Field, J. B., Bloom, G., Kerins, M. E., Chayoth, R. and Zor, U. (1975) J. Biol. Chem. 250, 4903–4910.
[6] Delbauffe, D. and Pavlovic-Hournac, M. (1976) FEBS Lett. 69, 59–62.
[7] Habhab, D., Bhalla, R. C. and Halmi, N. S. (1979) Biochim. Biophys. Acta 571, 233–243.

Table 3
Estimation of protein kinase activity (pmol ^{32}P . mg tissue^{-1} . 5 min^{-1}) in toxic nodule, perinodular region and intact tissue

Fraction	Tissue	Total	Type I	Type II	Type I / Type II
Cytosol	Control	43.83	29.76	14.07	2.12
	Perinodular	28.06	16.05	12.01	1.34
	Nodule	20.93	8.48	12.45	0.68
Particles	Control	4.66	3.77	0.89	4.24
	Perinodular	3.91	2.52	1.39	1.81
	Nodule	7.49	4.14	3.35	1.24

[8] Combest, W. L., Chiasson, R. B. and Haddock-Russell, D. (1979) Gen. Comp. Endocrinol. 40, 494–502.
[9] Costa, M., Gerner, E. W. and Russell, D. H. (1976) J. Biol. Chem. 251, 3313–3319.
[10] Costa, M., Gerner, E. W. and Haddock-Russell, D. (1978) Biochim. Biophys. Acta 538, 1–10.
[11] Haddox, M. K., Magun, B. E. and Haddock-Russell, D. (1980) Proc. Natl. Acad. Sci. USA 77, 3445–3449.
[12] Lee, P. C., Radloff, D., Schweppe, J. C. and Jungman, R. A. (1976) J. Biol. Chem. 251, 914–921.
[13] Conti, M., Geremia, R. and Monesi, V. (1979) Mol. Cell. Endocrinol. 13, 137–148.
[14] Eppenberger, U., Roos, W., Fabbro, D., Sury, A., Weber, J., Bechtel, E., Huber, P. and Jungmann, R. A. (1979) Eur. J. Biochem. 98, 253–259.
[15] Haddox, M. K., Roeske, W. R. and Haddock-Russell, D. (1979) Biochim. Biophys. Acta 585, 527–534.
[16] Byus, C. V., Chubb, J. M., Hustable, R. J. and Russell, D. H. (1976) Biochem. Biophys. Res. Commun. 73, 694–702.
[17] Roskoski, R. Jr, Ngau, P., Mettenburg, R. and Lund, D. D. (1978) Biochem. Biophys. Res. Commun. 82, 641–647.
[18] Podesta, E. J., Dufau, M. L., Solano, A. R. and Catt, K. J. (1978) J. Biol. Chem. 253, 8994–9001.
[19] Hunzicker-Dunn, H. and Jungmann, R. A. (1978) Endocrinol. 103, 431–440.
[20] Schwoch, G. (1978) Biochem. J. 170, 469–477.
[21] Schwoch, G. and Hilz, H. (1977) Eur. J. Biochem. 76, 269–276.
[22] Byus, C. V., Hayes, J. S., Brendel, K. and Haddock-Russell, D. (1979) Mol. Pharm. 16, 941–949.
[23] Guinovart, J. J. and Larner, J. (1980) Biochim. Biophys. Acta 631, 253–263.
[24] Handschin, J. C. and Eppenberger, U. (1979) FEBS Lett. 106, 301–304.
[25] Gharrett, A. J., Malkinson, A. M. and Sheppared, J. R. (1976) Nature 264, 673–675.
[26] Miyazaki, K., Miyamoto, E., Maeyama, M. and Uchida, M. (1980) Eur. J. Biochem. 104, 535–542.
[27] Delbauffe, D., Ohayon, R. and Pavlovic-Hournac, M. (1979) Mol. Cell. Endocrinol. 14, 141–155.
[28] Spaulding, S. W. and Burrow, G. N. (1972) Endocrinology 91, 1343–1349.
[29] Wilson, M. B. and Malkin, A. (1974) Can. J. Biochem. 52, 319–326.
[30] Leonard, J. L. and Rosenberg, L. L. (1977) Biochim. Biophys. Acta 484, 336–347.
[31] Pommier, J., Tourniaire, J., Deme, D., Chalendar, D., Bornet, H. and Nunez, J. (1974) J. Clin. Endocr. Metab. 39, 69–80.
[32] Pommier, J., Tourniaire, J., Rahmoun, B., Deme, D., Pallo, D., Bornet, H. and Nunez, J. (1976) J. Clin. Endocr. Metab. 42, 319–329.
[33] Abdelmoumene, N., Gavaret, J. M., Pommier, J. and Nunez, J. (1978) J. Mol. Med. 3, 305–318.