

ALTERED LEVELS OF CYCLIC NUCLEOTIDES, CYCLIC AMP PHOSPHODIESTERASE AND ADENYLYL CYCLASE ACTIVITIES IN NORMAL, DYSPLASTIC AND NEOPLASTIC HUMAN MAMMARY TISSUE

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1. Introduction

Recently, we have reported decreased cellular levels of cAMP-dependent protein kinase and cAMP-binding activity in neoplastic human mammary tissue [1]. Considering the sensitivity of the cAMP-protein kinase system to changes of cAMP, it was important to determine intracellular levels of this cyclic nucleotide and to identify changes of phosphodiesterase and adenylyl cyclase activities, the two enzyme systems which directly regulate the intracellular cAMP metabolism.

Recently, increased levels of cAMP were reported in human mammary carcinomas [2]. Our results show that neoplastic mammary tissues exhibit increased specific levels but decreased cellular levels of cAMP and cGMP. In addition, the cellular activities of adenylyl cyclase phosphodiesterase are significantly lower in neoplastic as compared with normal human breast tissues.

Enzymes: Phosphodiesterase, cyclic nucleotide phosphodiesterase (EC 3.1.4.c); Adenylyl cyclase, adenylyl cyclase (EC 4.6.1.1.)

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2. Materials and methods

Cyclic AMP, cGMP and phosphodiesterase were obtained from Sigma Chemical Company. Cyclic [³H]AMP (37 Ci/mmol) was purchased from New England Nuclear. ¹²⁵I-Labeled cyclic AMP and ¹²⁵I-labeled cyclic GMP were obtained from Schwarz/Mann (Orangeburg, NY).

Breast tissue specimens were obtained from patients during mastectomy, through biopsy (2–6 g tissue), and from patients undergoing plastic surgery. The specimens were frozen immediately after excision and stored at –70°C. For the various biochemical assays the breast tissue was pulverized in liquid nitrogen. For the cAMP-phosphodiesterase assay the powdered tissue was homogenized in 5 parts (w/v) of ice cold 10 mM Tris–HCl buffer, pH 7.4, containing 1.5 mM EDTA, 5 mM theophylline with a Polytron PT-20 (Brinkman Instr.). The homogenate was centrifuged for 90 min at 105 000 × g. The supernatant fraction was assayed for cAMP-phosphodiesterase activities according to Thompson et al. [3].

To assay adenylyl cyclase activity the powdered tissue was homogenized for 1 min in 20 parts (w/v) of ice-cold 10 mM Tris–HCl buffer, pH 7.4, containing 6 mM mercaptoethanol, using an Ultra Turrax (Janke and Kunkel, FRG) at position 6.5. The homogenate was filtered through 2 layers of nylon gauze and the filtrate was used for adenylyl cyclase assay, which was carried out according to Salomon et al. [4]. Protein was determined by the method of Lowry et al. [5].

Cyclic AMP was determined either by radioimmunoassay [6] or by the competitive binding assay [7] using a cAMP-binding protein isolated by DEAE-cellulose chromatography of calf ovarian cytosol [8]. The specificity for cAMP was tested by treatment of representative samples with phosphodiesterase (Sigma) which destroyed all of the binding activity.

Morphological characterization and histometry was carried out as previously described [1]. The tissues were divided in 5 categories: MI-F (simple dysplasia with extensive fibrosis), MI (simple dysplasia), MII (proliferative dysplasia), CA (primary carcinoma) and FA (fibroadenoma). MI-F was regarded as normal tissue [1].

3. Results

3.1. Cyclic AMP levels

Cyclic AMP was analyzed in 121 tissue specimens and the results are shown in fig.1A. Cyclic AMP per gram tissue was significantly ($p < 0.001$) higher in CA than in normal or dysplastic tissue. In fibroadenomas the cAMP concentrations were slightly lower ($p < 0.05$) than in carcinomas. It is of interest that in MI and MII approx. 20% of the tissue samples examined were responsible for the relatively large standard deviations. Also, 6 out of 24 tissue samples of the CA-group exhibited cAMP levels greater than 1200 pmol/g tissue.

3.2. Cyclic GMP-levels

In 70 tissue specimens cGMP (pmol/g tissue) was measured and the results are shown in fig.1B. In analogy to cAMP, the levels of cGMP were significantly elevated in carcinoma as compared to normal tissue ($p < 0.002$) and simple dysplasia ($p < 0.01$).

3.3. Phosphodiesterase

Phosphodiesterase activities of crude cytosol fractions measured at two substrate concentrations (6×10^{-8} and 1×10^{-6} M cAMP) are shown in table 1. At both substrate concentrations phosphodiesterase activities were significantly increased in carcinoma tissues as compared to MI-F. At the lower substrate concentration phosphodiesterase activity is significantly higher in MII, CA and FA.

3.4. Adenylyl cyclase

The adenylyl cyclase activities of mammary tissue at various stages of dysplasia (MI-F, MI, MII) was compared with 13 carcinomas (table 2). Specific adenylyl cyclase activities are significantly elevated in simple, proliferative dysplasia and carcinoma tissue as compared to normal tissue (MI-F).

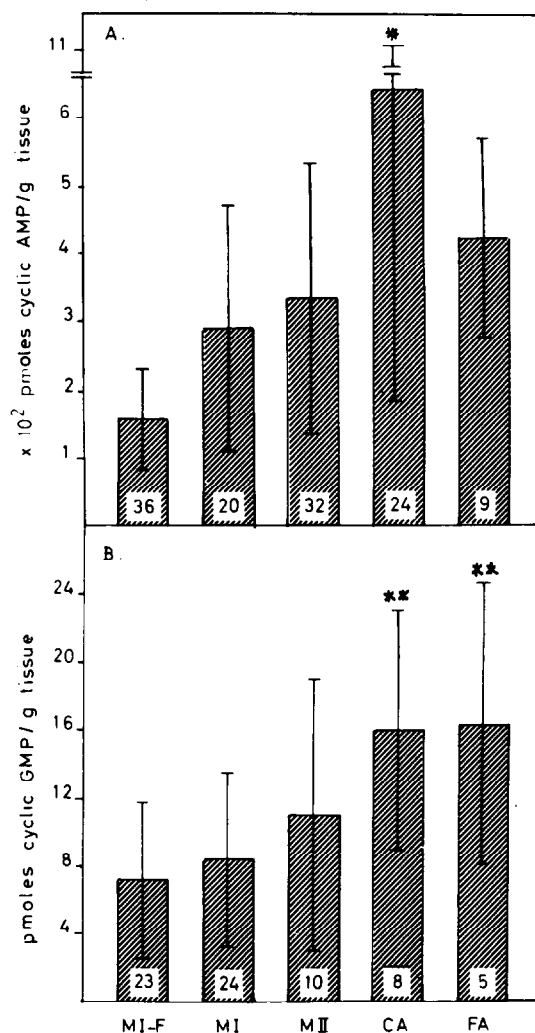


Fig.1. Cyclic AMP (A) and cGMP levels (B) in mammary tissue obtained from normal tissue (MI-F), simple (MI) and proliferative dysplasia (MI I), fibroadenoma (FA) and neoplastic tissue (CA). Each bar represents the mean ± SD. Number within bar indicates number of tissue specimens analyzed. *Significantly different ($p < 0.001$) as compared to MI-F **Significantly different ($p < 0.002$) as compared to MI-F.

Table 1
Cyclic phosphodiesterase activity in human breast tissue

| Tissue | Number of patients | Phosphodiesterase activity (pmol cAMP hydrolyzed/mg protein) | |
|---|--------------------|--|----------------------|
| | | 6×10^{-8} M cAMP | 10^{-6} M cAMP |
| Simple dysplasia with extensive fibrosis (MI-F) | 35 | 2.4 ± 0.8 (18) | 11.7 ± 6.7 (17) |
| Simple dysplasia (MI) | 43 | 2.0 ± 1.0 (13) | 10.0 ± 7.0 (30) |
| Proliferative dysplasia (MII) | 36 | 3.6 ± 1.4 (25) ^a | 13.7 ± 8.4 (11) |
| Carcinoma (CA) | 42 | 4.0 ± 1.4 (25) ^b | 21.5 ± 18.6 (13) |
| Fibroadenoma (FA) | 13 | 6.0 ± 1.5 (9) ^c | 13.8 ± 12.3 (4) |

^a $p < 0.02$;

^b $p < 0.005$;

^c $p < 0.001$ as compared to MI-F. Number of individual tissues examined in parentheses

The phosphodiesterase activity was measured in crude cytosol fractions at two different substrate concentrations (6×10^{-8} and 1×10^{-6} M cAMP). Each value is the mean \pm SD

3.5. Relative cellular levels of cAMP – and cGMP – phosphodiesterase and adenylyl cyclase

The above results are presented as specific enzymatic activities or cyclic nucleotide levels per unit weight of tissue protein and do not take into account the marked variations of cellular densities within the various histopathological groups. When the previously described histometric values [1] are considered the relative cellular levels of the cyclic nucleotides (fig.2), phospho-

diesterase and adenylyl cyclase (table 2) can be obtained. Such a correlation reveals markedly lower levels of cellular adenylyl cyclase activity in CA (table 2) and lower levels of cAMP (2–3-fold) and cGMP (4-fold) in CA and MII tissues (fig.2) as compared to normal tissue. Similarly the relative cellular phosphodiesterase activities were 3–4-fold lower in CA and MII than in normal tissue (fig.3).

Table 2
Adenylyl cyclase activity in human breast tissue

| Tissue | Number of patients | Adenyl cyclase activity | |
|---|--------------------|-------------------------------|--------------------|
| | | pmol cAMP formed/g tissue/min | rel. activity/cell |
| Simple dysplasia with extensive fibrosis (MI-F) | 8 | 316 ± 127 | 421 ± 221 |
| Simple dysplasia (MI) | 11 | 959 ± 590^a | 530 ± 254 |
| Proliferative dysplasia | 12 | 962 ± 658^a | 247 ± 128 |
| Carcinoma (CA) | 13 | 1727 ± 1535^a | 308 ± 247 |

^a $p < 0.01$ as compared to MI-F

The adenylyl cyclase assay was carried out with crude homogenate in the presence of 8×10^{-3} M NaF as effector [12]. Each value is the mean \pm SD. The adenylyl cyclase activity per cell was calculated by dividing the specific activity through the cellular density [1] of each individual histopathological group

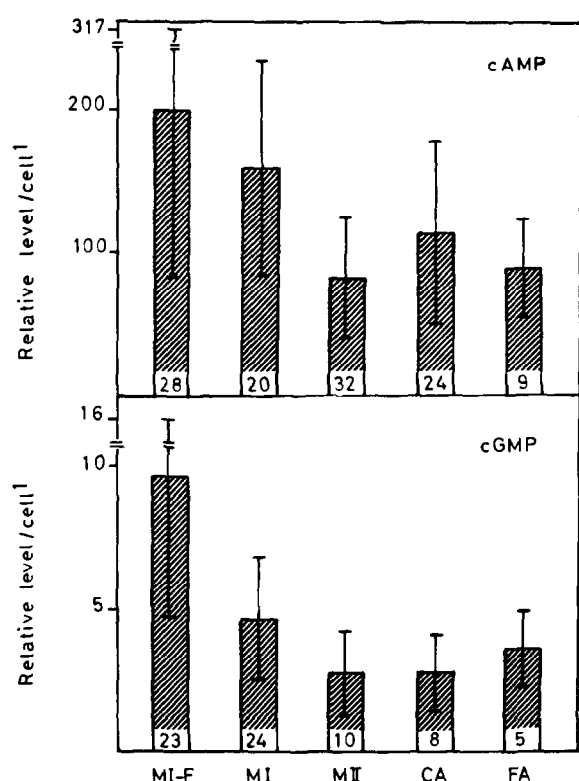


Fig.2. Relative cellular levels of cAMP and cGMP. The results were obtained after dividing the individual, specific cAMP and cGMP levels by the respective cellular density (MI-F, 0.75 ± 0.49 ; MI, 1.81 ± 0.64 ; MII, 3.89 ± 1.43 ; CA, 5.60 ± 2.29 ; FA, 4.59 ± 1.16 cells/1000 μm^2).

4. Discussion

The results presented indicate that cAMP and cGMP levels as well as phosphodiesterase and adenylyl cyclase levels are significantly elevated in neoplastic human breast tissue provided the values are compared per unit weight of tissue or per unit weight of cellular protein. However, these values do not take into account the relative cellular densities which are known to characterize the various morphological classes of human breast tissue [1]. When these biochemical data are expressed as relative cyclic nucleotide levels and enzymatic activities per cell, the neoplastic tissues are characterized by markedly lower cyclic nucleotide and enzymatic activities.

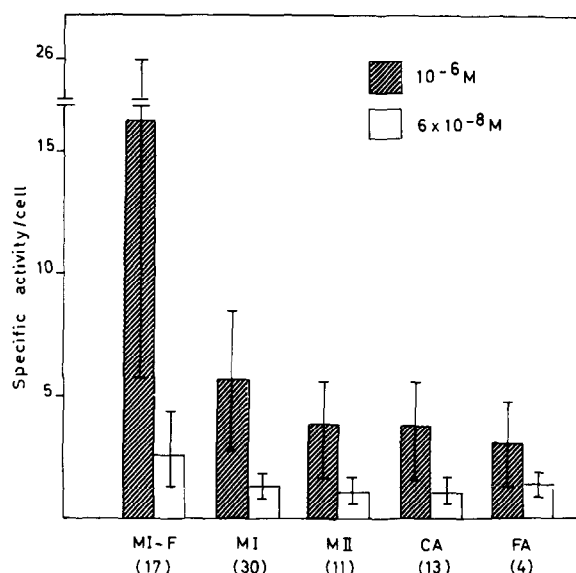


Fig.3. Relative cellular activity of cAMP phosphodiesterase. These cellular activities were calculated as described in fig.2. Number of individual tissues examined in parentheses.

In view of the changing morphological and biochemical characteristics of tumors, particularly changes of the cellular DNA and protein content, we believe that a correlation of cyclic nucleotide and enzymatic levels with the cellular density provides a meaningful way to compare and monitor biochemical changes during neoplastic transformation. Correlation of biochemical data with the cellular densities may, in fact, be used to resolve the controversy of previous findings in which both increases and decreases of cyclic nucleotide levels and of phosphodiesterase have been observed.

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