CONTENT AND METABOLISM OF CYCLIC AMP AT DIFFERENT STAGES OF GROWTH OF HEPATOMA 22a

T. I. Solntseva and A. K. Belousova

UDC 616.36-006-092.9-008.939.633.2

The intracellular content of cyclic 3',5'-adenosine monophosphate (AMP) and activity of adenylate cyclase and cyclic AMP phosphodiesterase were determined in the lag period and the exponential and stationary phases of growth of mouse hepatoma 22a. In the stage of transition of the tumor cells from the lag period to the exponential phase of growth, the intracellular cyclic AMP concentration was found to be reduced by half as the result of sudden activation of phosphodiesterase. Later the cyclic AMP content fell more slowly until the cells entered the stationary phase of growth. Since adenylate cyclase activity remained unchanged during growth of the hepatoma 22a, this suggests that an increase in phosphodiesterase activity is the signal for escape of the tumor cells from the resting period and their entry into the mitotic cycle.

KEY WORDS: *cyclic AMP content; adenylate cyclase; phosphodiesterase; mouse hepatoma 22a.*

The problem of the regulation of cell growth is nowadays at the center of attention of many investigators. It has recently been postulated that the central mechanism controlling cell multiplication is a change in the level of the cyclic nucleotides, especially cyclic AMP [4, 5, 13]. This has been shown chiefly in cultures of fibroblasts and lymphocytes [10]. The intracellular concentration of cyclic AMP is a function of the density of the cell population: in contact-inhibited fibroblasts the content of this nucleotide rises sharply, whereas addition of cyclic AMP to a culture of transformed fibroblasts leads to contact inhibition of growth [12]. It has been shown with cultures of leukemia L1210 cells that cyclic GMP and cyclic UMP are able to prolong the lag phase by 1.5-22 h depending on their concentration. Cyclic AMP, on the other hand, causes the tumor cells to enter the cycle immediately after its addition to the culture medium [2]. These observations suggest that the cellular cyclic AMP level is inversely proportional to the rate of proliferation. However, the data cited above were obtained in experiments *in vitro*. Data in the literature on cyclic AMP metabolism in hepatomas *in vivo* are very contradictory [10].

The object of this investigation was to study correlation between the intracellular cyclic AMP level and adenylate cyclase and cyclic AMP phosphodiesterase levels, on the one hand, and the rate of growth of hepatoma 22a on the other hand.

EXPERIMENTAL METHOD

Male C3HA mice aged 2 months were used. Hepatoma 22a (solid) was obtained by V. I. Gel'shtein in 1953 from a primary hepatoma induced in a mouse liver by o-aminoazotoluene. It is currently a rapidly growing carcinoma.

The incubation mixture (0.2 ml) for determination (15 min, 37°C) of adenylate cyclase activity contained: 1 mM ATP-^{3}H (about 1 μCi), 10 mM $MgCl_2$, 10 mM theophylline, 0.1% albumin, 10 mM NaF, 10 mM Tris-HCl, pH 7.4, and 1-1.5 mg protein of the homogenate. Before the end of incubation 0.02 ml of cyclic AMP (1 mg/ml) was added.

The incubation mixture for determination (5 min, 37°C) of cyclic AMP phosphodiesterase activity contained: 1 μM cyclic [^{3}H]AMP (about 0.15 μCi), 1 mM $MgCl_2$, 80 mM Tris-HCl, pH 8.0,

Laboratory of Biochemical Pharmacology, Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny Vol. 84, No. 10, pp. 472-474, October, 1977. Original article submitted April 7, 1977.

 0007-4888/77/8410-1480$07.50

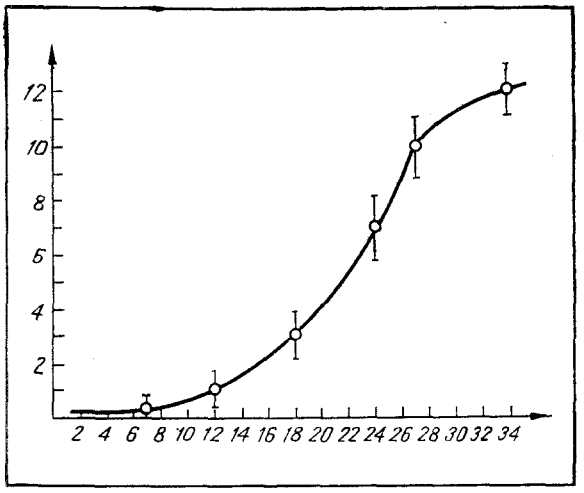

Fig. 1

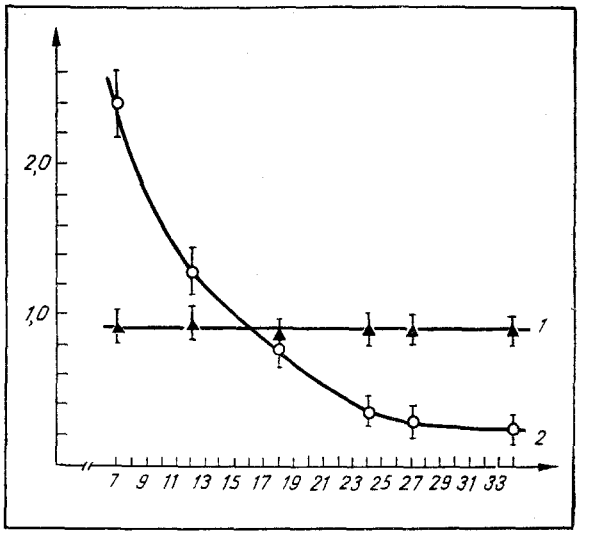

Fig. 2

Fig. 1. Kinetics of growth of hepatoma 22a. Abscissa, days after transplantation; ordinate, weight of tumor (in g).

Fig. 2. Intracellular content of cyclic AMP at different stages of tumor growth. 1) Liver of animal with tumor; 2) hepatoma 22a. Abscissa, days after transplantation; ordinate, cyclic AMP content (in pmoles/mg tissue).

and 100-200 μg protein of the homogenate. Before the end of incubation 0.01 ml of 5'-AMP (1 mg/ml) was added. Protein was added to the control tubes immediately before the reaction mixture was boiled.

The reaction was stopped by boiling, the tubes were centrifuged for 10 min at 3000g, the supernatant was applied to a Silufol UV 254 plate, and ascending chromatography was carried out in two successive systems of solvents: isopropanol—ethyl acetate—25% ammonia (59:25:16) and n-butanol—acetic acid—water (5:1:1). Radioactivity was determined in toluene—Triton scintillator in the presence of 0.025 M $MgSO_4$ [6]. The intracellular cyclic AMP level was determined by the standard "Cyclic AMP Assay Kit." Tissue extracts were prepared as described previously [3]. Protein was determined by Lowry's method [7].

EXPERIMENTAL RESULTS

To investigate the kinetics of growth of hepatoma 22a the tumors were weighed on the 7th, 12th, 18th, 24th, 27th, and 34th days after transplantation. Each time tumors were weighed from 4-6 animals. As Fig. 1 shows, the end of the lag phase of hepatoma 22a occurred on the 7th day after transplantation, and tumor cells emerged from it on the 8th and 9th days. The lag period was followed by the exponential phase of growth, and then by the stationary phase, when the weight of the hepatoma reached 12 ± 0.8 g. The shortest time for the tumor to double its mass was observed in the segment of the curve from the 7th to the 12th day after transplantation. Consequently, the maximal rate of growth corresponded to the transition of the tumor cells from the lag period into the exponential phase of growth, in agreement with previous observations [1].

The first measurement of the intracellular cyclic AMP concentration was made at the end of the lag phase (Fig. 2). The content of the nucleotide at this period was 2.4 ± 0.2 pmoles/mg tissue (2.7 times higher than in the liver of the animal with the tumor). In the phase of exponential growth (12th day after transplantation) a sharp decrease in the cyclic AMP concentration (by half) was observed and compared with the initial level, and this was followed by a more gradual decrease (down to 0.33 ± 0.03 pmole/mg tissue) in the stationary phase of tumor growth. The content of the nucleotide in the liver of the animal with the tumor remained virtually unchanged. A high rate of proliferation of hepatoma 22a cells after the resting period is thus evidently the result of a decrease in the intracellular concentration of cyclic AMP. The content of this nucleotide in the cell is controlled by the relative activities of the enzymes of its synthesis (adenylate cyclase) and hydrolysis (cyclic AMP phosphodiesterase). The next task was therefore to discover which enzyme of cyclic AMP metabolism is responsible for the entry of the tumor cells into the mitotic cycle.

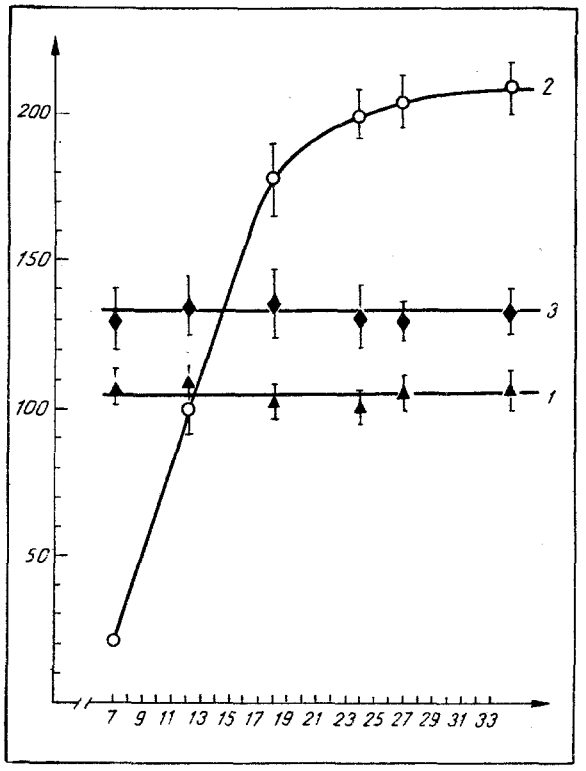

Fig. 3. Changes in activity of enzymes of cyclic AMP metabolism at different stages of tumor growth: 1) cyclic AMP phosphodiesterase in mouse liver, 2) in hepatoma 22a; 3) adenylate cyclase in hepatoma 22a and in mouse liver. Abscissa, days after transplantation; ordinate, cyclic AMP activity (in pmoles/mg protein/min).

The adenylate cyclase activity remained unchanged at the different stages of growth of hepatoma 22a and was virtually indistinguishable from the control. Activity of cyclic AMP phosphodiesterase in the lag phase was only one-fifth of that in the liver, and during the transition of the tumor cells from the resting phase to the exponential phase of growth (corresponding to the maximal rate of proliferation), activity of the enzyme increased sharply and continued to rise gradually until the last day of observation (Fig. 3). The entry of the tumor cells into the mitotic cycle (the transition from the lag to the exponential phase) is thus evidently regulated by a change in the activity of cyclic AMP phosphodiesterase. If it is accepted that this enzyme plays a trigger role in the regulation of cell division, attempts by clinicians to inhibit the "uncontrollable" growth of tumor cells by the use of chemotherapeutic inhibitors of cyclic AMP phosphodiesterase are justified.

LITERATURE CITED

1. L. B. Klempner, "Kinetics of cell proliferation of transplantable mouse hepatomas," Author's Abstract of Candidate's Dissertation, Moscow (1975).
2. A. Bloch, G. Dutschman, and R. Maue, Biochem. Biophys. Res. Commun., 59, 955 (1974).
3. A. G. Gilman, Proc. Nat. Acad. Sci. USA, 67, 305 (1970).
4. M. L. Heidrich and W. L. Ryan, Cancer Res., 30, 376 (1970).
5. G. S. Johnson, R. M. Friedman, and I. Pastan, Proc. Nat. Acad. Sci. USA, 68, 425 (1971).
6. J. J. Keirns, M. A. Wheeler, and M. W. Bitensky, Anal. Biochem., 61, 336 (1974).
7. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
8. S. M. Naseem and V. P. Hollander, Proc. Am. Assos. Cancer Res., 15, 139 (1974).
9. J. Otten, G. S. Johnson, and I. Pastan, Biochem. Biophys. Res. Commun., 44, 1192 (1971).
10. I. H. Pasten, G. S. Johnson, and W. B. Anderson, Annu. Rev. Biochem., 44, 491 (1975).
11. A. Rein, R. A. Carchman, G. S. Johnson, et al., Biochem. Biophys. Res. Commun., 52, 899 (1973).
12. J. R. Sheppard, Proc. Nat. Acad. Sci. USA, 68, 1316, (1971).
13. J. R. Sheppard, Nature (London), New Biol., 236, 14 (1972).