EFFECT OF DIBUTYRYL DERIVATIVES OF CYCLIC NUCLEOTIDES ON TOTAL DNA AND PROTEIN SYNTHESIS IN RAT FETAL HEPATOCYTE CULTURES

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UDC 612.352.3-053.1.085.23.044:577.123.3

KEY WORDS: analogs of cyclic nucleotides; fetal hepatocyte culture; RNA and protein synthesis

The writers showed previously that dibutyryl derivatives of cyclic adenosine monophosphate (Bt_2cAMP) and guanosine-3′,5′-monophosphate (Bt_2cGMP) have a biphasic effect on primary cultures of liver cells from rat fetuses and young rats aged 3 weeks during short-term (4 h) incubation these compounds stimulate albumin production, whereas during long-term (20 h) incubation, and in a high concentration (10^{-3} M) the depress this indicator of hepatocyte function [3]. The inhibitory action of Bt_2cAMP which we observed, at least in rat hepatocytes, could be connected with inhibition of synthesis of total protein, for cAMP and its analogs have a direct inhibitory effect on the intensity of this process, starting with the 12th-16th day after birth [7]. Meanwhile effects of cAMP analogs on total RNA synthesis in the liver have virtually not been studied. Butyrate formed during metabolism of Bt_2cAMP in the liver [9] itself possesses appreciable biological activity, due to inhibition of histone deacetylation [10]. Moreover, the effect of a high butyrate concentration (5 mM) on mature hepatocytes in culture, as a function of time, has been shown to be biphasic in character [11]. The biphasic effect which we observed previously, at least for Bt_2cAMP, on albumin production by hepatocytes [3], could therefore be mediated not by the cyclic nucleotide itself, but by butyrate.

The aim of this investigation was to study the effect of dibutyryl derivatives of cyclic nucleotides and of butyrate on total RNA and protein synthesis in fetal hepatocytes and also to compare the effects of these compounds on albumin production.

EXPERIMENTAL METHOD

Experiments were carried out on primary cultures of rat fetal liver cells, grow in selective medium to which barbiturate and glucocorticoid were added with a view to enhancing the viability and functional activity of the hepatocytes [8]. A suspension. of isolated rat fetal liver cells was obtained as described previously [1-3]. The cell suspension was seeded into 24-well plastic panels, treated beforehand with bovine serum, and the cells were grown for 2-4 days in medium of the following composition: 90% medium 199 (or RPMI-1640), containing 3.75 mM barbital sodium, 2.5 μg/ml cortisol, 10 mM HEPES, 2 mM glutamine, 50 U/ml benzylpenicillin, and 10% bovine serum. The cells were then cultured in the same medium but without barbiturate and glucocorticoid. Incubation with the test agents, taken in a concentration of 10^{-3} M, was carried out on the 8th-9th day in medium 1% bovine serum. Samples were taken 2 h before the end of incubation to determine albumin, and ^{3}H-uridine and ^{14}C-leucine were added. Synthesis of total RNA and intracellular protein was studied by determining incorporation of the labeled precursors into acid-insoluble cell material, and albumin production was determined by a homologous radioimmunologic method; the protein concentration in the cultures was measured colorimetrically [1-3]. A mother solution of butyrate was obtained by neutralizing a solution of butyric acid with the equimolar quantity of NaOH. The results were subjected to statistical analysis by Student's t-test.

(Presented by Academician of the Academy of Medical Sciences of the USSR Yu. A. Pankov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 5, pp. 478-480, May, 1991. Original article submitted May 25, 1990.

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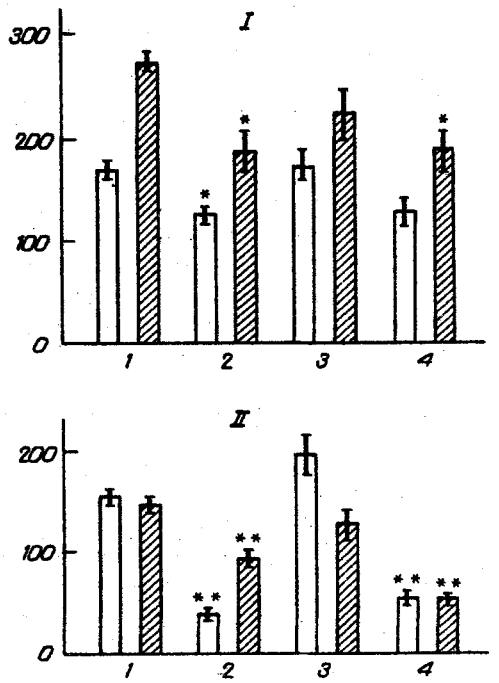

Fig. 1. Effect of dibutyryl derivatives of cyclic nucleotides and sodium butyrate on total RNA and protein synthesis in rat fetal liver cell cultures (n = 3-6). Incubation for 6 h (I) and 24 h (II). Columns: shaded — protein synthesis, unshaded — RNA synthesis, concentration of all test agents 10^{-3} M. Ordinate, incorporation of labeled precursors (in cpm/μg protein). 1) Control, 2) Bt_2cAMP, 3) Bt_2cGMP, 4) butyrate. *p < 0.01, **p < 0.001.

TABLE 1. Effect of Dibutyryl Derivatives of Cyclic Nucleotides and of Sodium Butyrate on Albumin Production by Rat Fetal Liver Cells (concentration of all test agents 10^{-3} M)

Group	Albumin in medium, ng/μg protein	
	after 4 h	after 22 h
Control	1,870±0,027 (4)	4,256±0,247 (5)
Bt_2cAMP	4,203±0,566 (4)*	8,229±0,709 (3)*
Bt_2cGMP	4,118±0,238 (4)**	5,065±0,819 (3)
Sodium butyrate	2,108±0,341 (4)	5,130±0,764 (5)

Legend. Number of observations in parentheses. *p < 0.01, **p < 0.001.

EXPERIMENTAL RESULTS

In the first experiment (Fig. 1, Table 1) incubation with the test agents lasted 6 h (I). In this case Bt_2cAMP stimulated albumin production but definitely inhibited RNA and protein synthesis. Sodium butyrate did not alter albumin production but had a tendency to inhibit RNA synthesis and it significantly inhibited protein synthesis in the cultured hepatocytes. Bt_2cGMP stimulated albumin production and had a tendency to stimulate RNA synthesis, but did not change the intensity of total protein synthesis.

The results showed that dibutyryl derivatives of cyclic nucleotides and sodium butyrate have most frequently opposite effects on total RNA and intracellular protein synthesis, on the one hand, and on albumin production, on the other hand. These results can be fully explained by data in the literature indicating that intracellular and secreted proteins are synthesized in hepatocytes on free and membrane-bound polysomes respectively [5] and that, consequently, synthesis of these proteins is evidently regulated independently.

Recently published results of studies conducted on neuroblastoma cells in culture showed that Bt_2cAMP (10^{-3} M), but not butyrate ($2 \cdot 10^{-3}$ M), induces axon formation, whereas Bt_2cAMP and butyrate reduce acetylcholinesterase activity in the cells [12]. The opposite nature of the effects of Bt_2cAMP and butyrate on the same parameters of cells in culture, whereas at the same time these agents have similar action on other parameters, revealed both by the investigation cited above and our own work, will be noted. In our view this indicates that a trivial explanation cannot be found for the effects or Bt_2cAMP, namely through the action of butyrate released by intracellular hydrolysis of Bt_2cAMP. Evidence of this interpretation is also given by our data for Bt_2cGMP, although this analog is most probably degraded in the same way as Bt_2cAMP [9] to the monobutyryl derivative and butyrate by the action of a nonspecific esterase, rupturing the $O^{2'}$-ester bond, and the effects of Bt_2cGMP differ cardinally from those of Bt_2cAMP. In our view a particularly characteristic feature is that Bt_2cGMP has no significant effect on total RNA and protein synthesis despite the marked inhibitory action of Bt_2cAMP on these biosynthetic processes.

In some biological systems cAMP and cGMP have opposite actions, maintaining the balance of cell functional activity [6], in the same way as glucagon and insulin maintain the balance between catabolic and anabolic processes in hepatocytes. There is reason to suppose that at least some effects of glucagon and insulin on hepatocytes are mediated through cAMP and cGMP respectively [4]. Our own studies showed that insulin stimulates total RNA and protein synthesis in cultures of liver cells from fetuses and young rats [1], whereas glucagon inhibits protein synthesis, at least in hepatocytes of young rats [2]. Consequently, the effects of insulin and glucagon on macromolecular biosynthesis which we observed in hepatocytes [1, 2] can hardly be effected purely by changes in the intracellular cyclic nucleotide levels.

It can be concluded from the results of this investigation that a catabolic action of the cAMP analog on biosynthesis of total RNA and protein can be demonstrated in rat fetal liver cells cultured in selected medium, but the observed anabolic effect of the cGMP derivative on RNA synthesis is nothing more than a tendency. In our view the catabolic action of the CAMP analog characteristically does not affect production of a secreted protein, namely albumin. This evidently indicates an important physiological role for albumin as a polyfunctional protein, essential for the body even in increased amounts in certain catabolic states. As was pointed out above, a possible mechanism enabling the cells in a state of catabolism to avoid a decrease in production of secreted proteins, may be the compartmentalization of synthesis of intracellular proteins and proteins intended for export on free and membrane-bound; polysomes respectively [5]. It also follows from the results that the effects of dibutyryl derivatives of cyclic nucleotides are not reproduced by butyrate, at least on albumin production by fetal hepatocytes.

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