OPPOSITE EFFECTS OF CYCLIC AMP AND ITS DIBUTYRYL DERIVATIVE ON GLYCOGEN LEVELS IN HELA CELLS

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SUMMARY:

In HeLa S3 cells, $N^6,O^{2'}$-dibutyryl-3',5'-cyclic-AMP (DBcAMP) leads to a decrease in glycogen content within two hours. This effect is reinforced by theophylline and counteracted by insulin and imidazole. In contrast to DBcAMP, 3',5'-cyclic AMP (cAMP) itself acts like insulin by increasing the glycogen content. It is synergistic with imidazole and insulin, and counteracts the theophylline effect partially. When added together, the dibutyryl derivative completely abolishes the anabolic action of cAMP on glycogen content.

INTRODUCTION:

cAMP has been implicated as an intracellular mediator of a large number of hormonal effects (cf. 1). Its fat-soluble derivative DBcAMP (2) proved to be superior to cAMP in imitating hormonal actions in many systems (3 - 8).

Insulin decreases the intracellular level of cAMP in fat pads (9) and liver (10). It counteracts the effects of cAMP on phosphorylated compounds in fat cells (11), and antagonizes the action of DBcAMP on the metabolism of surviving rat diaphragm (12). Imidazole can imitate the action of insulin by phosphodiesterase stimulation (13 - 15), while theophylline increases cAMP concentration by inhibition of phosphodiesterase (15 - 16).

When we observed by chance that insulin is able to stimulate glycolysis in HeLa S3 cells (17), experiments were performed to analyze the response of this established tumor cell line to hormonal

stimuli and to cAMP. While DBcAMP produced effects as expected, cAMP did not.

METHODS:

HeLa S3 monolayer cultures were propagated on Roux bottles (v = 800 ml) in modified Joklik medium (Grand Island Biol. Comp., N.Y.) fortified with 5% calf serum. Usually 3-5 x 10^7 cells per flask were seeded, and medium was changed after 24 hours. Substances dissolved in isotonic NaCl or water were added one day after seeding together with fresh medium and usually left for 24 hours. Control cultures received an equal amount of isotonic NaCl or water. Cells were harvested by detaching from the glass surface with the aid of 3 ml 0.25% trypsin dissolved in buffered isotonic salt solution and a

TABLE I

Antagonistic effects of dibutyryl cAMP and insulin on glycogen content

ADDITIONS	GLYCOGEN CONTENT nmoles 'glucosyl'/10^6 cells	%
none	46.4 ± 3.2	100 %
DBcAMP 7 x 10^{-4} M	29.3	63 %
DBcAMP 1 x 10^{-3} M	14.9 ± 1.7	32 %
insulin 2 x 10^{-6} M	71.0 ± 1.6	153 %
theophylline 3 x 10^{-3} M	29.2 ± 3.5	63 %
imidazole 3 x 10^{-3} M	62.2 ± 6.2	134 %
DBcAMP (10^{-3}M) + insulin	20.5 ± 1.6	44 %
DBcAMP (10^{-3}M) + theophylline	6.4 ± 1.3	14 %
DBcAMP (10^{-3}M) + imidazole	9.0 ± 0.1	19 %
insulin + theophylline	30 .2 ± 0.5	65 %
insulin + imidazole	58 .4 ± 5.7	126 %

4.7 x 10^7 cells per flask were incubated with the above additions together with new medium and analyzed after 24 hours. For further details see 'methods'. Typical experiments with mean values from two separate determinations.

'policeman', and rinsing two times with 3 ml medium. After homogenization with a syringe, cells were counted, and an aliquot of the suspension (ca. 5 x 10^7 cells) was centrifuged off. The cell pellet was digested with 0.50 ml 4 N KOH (20 min at 100°), followed by hydrolysis in 2.6 N HCl (60 min at 100°). Glucose was then determined enzymatically acc. to SLEIN (18).

Cyclic nucleotides were obtained from Fa. Boehringer u. Soehne, Mannheim, Germany. cAMP and DBcAMP were pure on the basis of adenine and P determinations as well as by chromatographic and spectral analysis.

RESULTS AND DISCUSSION:

Insulin, when added to HeLa S3 monolayer cultures, leads to an increase in glycogen content of the cells (table 1) (and also to a stimulation of glycolysis (17)). The dibutyryl derivative of cyclic

TABLE II

Stimulating action of cAMP on glycogen content and additive effect with insulin

ADDITIONS	GLYCOGEN CONTENT nmoles 'glucosyl'/10^6 cells	%
none	38.5 ± 2.6	100 %
cAMP 3 x 10^{-4} M	52.0	135 %
insulin 2 x 10^{-4} M	61.4	160 %
theophylline 3 x 10^{-3} M	21.1	55 %
imidazole 3 x 10^{-3} M	64.0	166 %
cAMP + insulin	75.9	197 %
cAMP + theophylline	32.0	83 %
cAMP + imidazole	65.0	169 %

4 x 10^7 cells per flask were incubated with the additions as indicated. For further details see legend to table I and 'methods'.

AMP (DBcAMP) acts in an opposite manner, as does theophylline, while imidazole imitates insulin.
Combination experiments reveal antagonistic effects of DBcAMP or of theophylline to the action of insulin and of imidazole, resp. (table 1).

While these observations are in accordance with the current hypothesis of insulin acting as an antagonist to cellular cAMP, it is not supported by the results obtained with cAMP itself. As shown in table 2, cAMP - in clear contrast to its dibutyryl derivative - acts like insulin and imidazole by elevating glycogen levels. Combination experiments produce additive effects with insulin and imidazole, while theophylline suppresses the action of cAMP on glycogen levels.

TABLE III

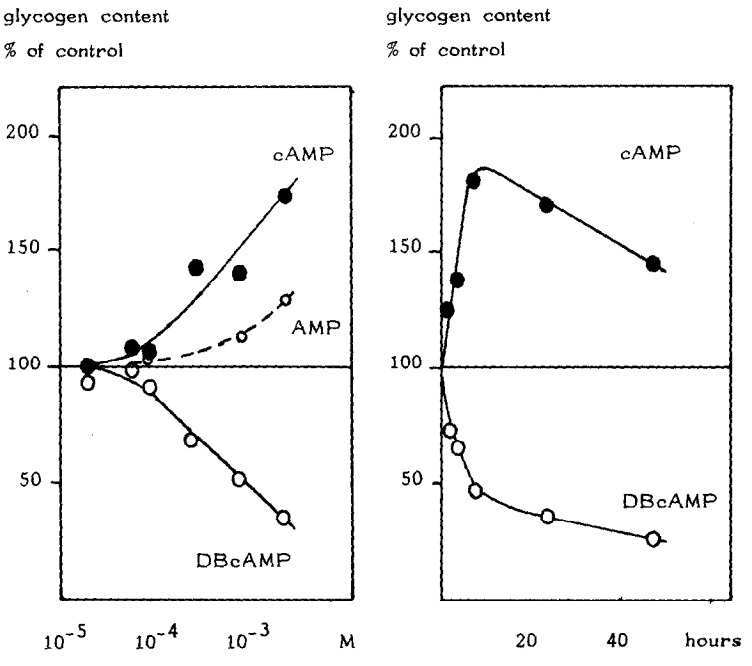
Antagonistic effects of cAMP and DBcAMP on glycogen content

ADDITIONS		CELL PROLIFERATION +)	GLYCOGEN CONTENT ++)	
none		1.34 ± 0.01	64.4 ± 7.9	100 %
cAMP	1×10^{-3}	1.06 ± 0.08	95.9 ± 4.4	149 %
DBcAMP	1×10^{-3}	1.18 ± 0.03	29.5 ± 3.0	40 %
cAMP + DBcAMP		0.97 ± 0.01	27.2 ± 3.0	42 %

4.4×10^7 cells per flask were incubated with the additions as indicated. Mean values from two cultures each.

+) Increase in cell number x 10^7/ flask

++) nmoles 'glucosyl'/10^6 cells



a) concentration dependency b) time dependency

Figure 1. Opposite effects of cAMP and its dibutyryl derivative. In a), cells were exposed to the nucleotides at 1 x 10^{-3} M final concentration for 24 hours. Glycogen content of control cells was 45.5 ± 2.9 nmoles 'glucosyl'/10^6 cells. - in b), all values are based on controls of a corresponding age after seeding.

The effects of both nucleotides, though opposite in direction, exhibit a similar dependency on concentration and time (fig. 1). Less than two hours after incubation are enough to give significant alterations in glycogen content. 5'-AMP at ten times higher concentrations acts like cAMP.

When combined, DBcAMP completely suppresses the action of cAMP under the conditions employed (table 3). There is no correlation to changes in cell proliferations. Both nucleotides tend to decrease cell proliferation. In this respect, they act synergistically. It should be mentioned though, that dense monolayers (with a low proliferation rate) respond more effectively to insulin than do cultures which permit a logarithmic growth rate (17).

The results obtained with this established tumor cell line show that it is still able to respond to hormonal stimuli. Insulin even at a concentration of 3×10^{-7} M produces significant augmentation of glycogen content. DBcAMP also leads to a 'normal' and expected response as do theophylline and imidazole resp. The action of cAMP, however, is hardly understood. It is not its insulin-like effect per se which does not fit in. There are other reports on insulin-like actions of cAMP (19-21). Rather, the opposite and antagonistic action of DBcAMP and cAMP in the HeLa system poses a challenge to

TABLE IV

The stimulating effect of various cyclic and non-cyclic nucleotides on glycogen levels as contrasted to mono- and dibutyryl-cAMP

ADDITIONS		GLYCOGEN CONTENT per 10^6 cells
none	-	100
AMP	1×10^{-3} M	130 ± 20
ATP	1×10^{-3} M	167
NAD	1×10^{-3} M	234 ± 30
cAMP	1×10^{-3} M	188 ± 18
cCMP	5×10^{-4} M	109
2',3'-cCMP	5×10^{-4} M	246 ± 22
DBcAMP	5×10^{-4} M	66 ± 8
MBcAMP	5×10^{-4} M	87 ± 2

Determinations were made 24 hours after exposure to 'additions'. Mean values from two experiments. Absolute values of the controls: 21.4 ± 2.6 nmoles 'glucosyl'/10^6 cells. MBcAMP = Monobutyryl-3',5'-cyclic AMP; cCMP = 3',5'-cyclic CMP; 2',3'-cCMP = 2',3'-cyclic CMP.

standard interpretations. It is not explained by dualistic effects of DBcAMP as observed in adipocytes, where it inhibits glycerol release at low, and stimulates it at higher concentrations (19): The nucleotides do not show any inversion of their effects on glycogen levels at concentrations between 3×10^{-5}M - 3×10^{-3}M.

cAMP may exert its action at the cell surface, possibly by inhibiting adenyl cyclase in analogy to the nucleotide inhibition of adenyl cyclase observed in fat cell membranes (22). In contrast, the fat-soluble dibutyryl derivative should penetrate the membranes and act as a substitute of intracellular cAMP. Two observations favor such an interpretation: a) Monobutyryl-cAMP is considerably less effective than DBcAMP in decreasing glycogen content. b) Several nucleotides other than cAMP (cyclic and non-cyclic and including 5'-AMP) are able to increase glycogen content (table IV).

NOTE: After completion of the manuscript, a very recent paper of KITABCHI and co-workers (Biochim.Biophys.Res.Commun. 39,1065(1970) came to our attention. It shows insulin-like effects of cAMP in fat cells and an opposite action of dibutyryl cAMP. The interpretation, however, that "the action of insulin may be mediated by different cyclic nucleotides resembling c-TMP" may not be valid. At least in our system, non-cyclic nucleotides like AMP, ATP and NAD as well as 2',3'-cCMP are as effective or more effective than cAMP in imitating insulin (table IV).

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