INHIBITION OF DNA SYNTHESIS IN HAMSTER CHEEK POUCH TISSUE IN ORGAN CULTURE BY DIBUTYRYL CYCLIC AMP AND A HOMOLOGOUS EXTRACT

Robert W. Teel

Department Physiology, Pharmacology and Biophysics
School of Medicine
Loma Linda University
Loma Linda, California 92354

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SUMMARY: Hamster cheek pouch tissue cultured with 10^{-4}M dibutyryl cyclic AMP exhibits inhibition of the rate of DNA synthesis. When tissue is cultured with dibutyryl for different time periods throughout the 18 hours of incubation, the effect on the rate of DNA synthesis is very similar. A combination of dibutyryl cyclic AMP and an epithelial cell extract from hamster cheek pouch tissue containing a natural epithelial chalone, appears to have an additive effect on the inhibition of the DNA synthesis rate.

A hypothesis proposed for the control of cell division suggests that each cell has a specific growth stimulatory substance that remains within the cell and an inhibitory substance that can escape (1). Factors have been found which stimulate cell division (2, 3), while others inhibit (4-6). An extract from hamster cheek pouch epithelial cells inhibits DNA synthesis in pouch tissue (7). Epinephrine facilitates the inhibitory effect of an epidermal chalone on epidermal cell proliferation (5) and it has been shown that epinephrine stimulates an increase in adenosine 3', 5'-cyclic monophosphate levels in hamster epidermis (8).

This is a report of the effect of a cyclic AMP derivative, dibutyryl cyclic AMP, on the rate of DNA synthesis in hamster cheek pouch tissue alone and in combination with an epithelial extract known to inhibit DNA synthesis in hamster cheek pouch tissue (7).

MATERIALS AND METHODS

The methods for organ culture of hamster cheek pouch tissue have been described (8). Cultures are maintained for 18 hours in the presence of 10^{-4}M dibutyryl cyclic AMP (Sigma) and 10^{-3}M theophylline (Sigma). The last 6 hours of culture includes an exposure of the tissues to H^3-thymidine (New England Nuclear), 1 μc/ml of culture medium. The procedure for determining the DNA synthesis rate has been reported (8). Tissue samples are counted in a Beckman LS-250 liquid scintillation counter and the DNA synthesis rate recorded as the average number of cpm per 2 mm^2 piece of cultured tissue (n=6).

Preparation of the epidermal cell extract has been given (7). Tissues are cultured in the presence of the desired concentration of epidermal cell extract with and without 10^{-4}M dibutyryl cyclic AMP.

Table 1. The effect of 10^{-4}M dibutyryl cyclic AMP on the rate of DNA synthesis in the hamster cheek pouch grown in organ culture.

Treatment period[a]	Average	% Controls	S.E.
Controls	3594[b]	-	476
Full 18 hours	2730	76	506
First 6 hours	2816	78	361
First 12 hours	2973	83	575
Middle 6 hours	2766	77	371
Last 12 hours	2802	78	360
Last 6 hours	2822	78	364

a. All cultures were incubated in medium containing H^3-thymidine (1 μc/ml) the last 6 hours and counted in a liquid scintillation counter.

b. Each value is the number of cpm per 2 mm^2 piece of cultured tissue (n=6).

RESULTS

Hamster cheek pouch tissue cultured with dibutyryl cyclic AMP exhibits significant inhibition in the rate of DNA synthesis at 10^{-2}, 10^{-3}, and 10^{-4}M concentrations. When cultures are incubated with a 10^{-4}M concentration of dibutyryl cyclic AMP for different periods during the 18 hour incubation period, there appears to be no significant correlation between the period of exposure to dibutyryl and the inhibitory effect on the rate of DNA synthesis (Table 1). When various concentrations of hamster cheek pouch epithelial cell extract are combined with 10^{-4}M dibutyryl cyclic AMP, the effect on rate of DNA synthesis in the hamster cheek pouch appears to be additive (Fig. 1).

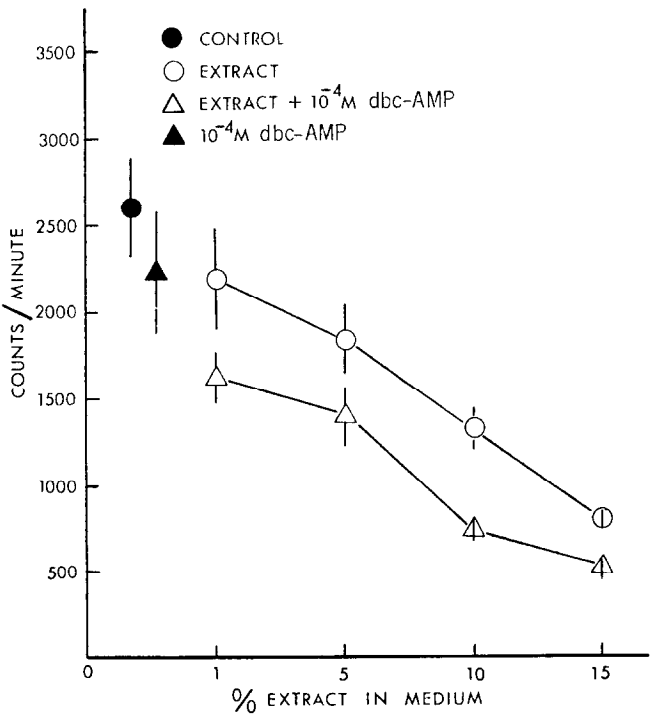

Fig. 1. The effect of 10^{-4}M dibutyryl cyclic AMP and hamster cheek pouch epithelial cell extract separately and in combination on the rate of DNA synthesis in hamster cheek pouch tissue cultures. H^3-thymidine was added to each culture 6 hours before the experiment was terminated. The vertical lines at each point represent the standard error of the mean (n=6).

DISCUSSION

Dibutyryl cyclic AMP has been shown to inhibit the growth of a number of cell lines (10, 11). The association of epinephrine with the inhibitory effect of an epidermal chalone and with cyclic AMP levels in epidermis (5, 8) suggests that cyclic AMP and the inhibitory substance are related. The numerous reports of the association of cyclic AMP and cell growth prompted me to implicate cyclic AMP in the control of cell proliferation. I hypothesized that the inhibitory effect of cyclic AMP is related to the synthesis of the inhibitory factor (s) isolated from hamster cheek pouch tissue and that increasing the endogenous level of cyclic AMP in cells would greatly enhance the inhibition of DNA synthesis by the epithelial extract.

Table 1 tabulates data showing the effect of 10^{-4}M dibutyryl cyclic AMP on the rate of DNA synthesis in hamster cheek pouch tissue. Inhibition occurs but the period of treatment whether for the full 18 hours of culture or for only part of it, does not seem to produce differences in the degree of inhibition. I had hoped that a specific part of the 18 hour culture period would exhibit greater sensitivity to the effects of dibutyryl than other parts. This would have indicated that some particular part of the cell cycle is sensitive.

The inhibitory effects of 10^{-4}M dibutyryl cyclic AMP and that of four different concentrations of extract on DNA synthesis rates in hamster cheek pouch tissue is shown (Fig. 1). The progressive inhibitory effect with increased amounts of extract supports previous data (7). When 10^{-4}M dibutyryl and extract are used in combination, the degree of inhibition appears to be additive. Data in Fig. 1 does not support my original hypothesis and apparently the inhibitory effect of dibutyryl cyclic AMP and that of the epithelial extract are independent.

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