Mediation of the Mitogenic Action of Growth Hormone by Adenosine 3′5′-Monophosphate (Cyclic AMP)[1] (34226)

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(Introduced by H. J. Morton)
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Growth hormone strongly stimulates the proliferation of thymic lymphocytes (thymocytes), and lymphoid cells in general, both *in vivo* and *in vitro* (1–6). Furthermore, this hormone increases the mitotic activity of thymocytes in the same indirect way as vasopressin and the parathyroid hormone; all three hormones simply facilitate the primary mitogenic action of calcium ions (5, 6). Since vasopressin and parathyroid hormone now appear to produce their various physiological effects by stimulating the intracellular formation of a common mediator (an intracellular 'messenger'), adenosine 3′5′-monophosphate (cyclic AMP) (7, 8), we suspected that this cyclic nucleotide might also mediate the mitogenic action of growth hormone.

Materials and Methods. To investigate this possibility, thymus tissue was removed from albino, male, specific-pathogen-free rats (bred in this laboratory) and thymocyte suspensions (containing 2×10^8 cells/ml) were prepared as previously described (9). The suspended cells were incubated at 37° (while contained in roller tubes revolving at 40 rpm) usually in a glucose–salts medium containing 5.5 m*M* glucose, 0.6 m*M* $CaCl_2$, 5.0 m*M* KCl, 1.0 m*M* $MgSO_4$, 120 m*M* NaCl, 5.0 m*M* Na_2HPO_4 and 5.0 m*M* tris (hydroxymethyl) aminomethane (Tris) buffer (pH 7.2). In some experiments, a much more complex medium (MAC-1) was used which consisted of the glucose–salts medium plus all of the vitamins, amino acids, nitrogenous bases, and supplementary growth factors of the synthetic tissue culture medium 199 (10). The mitotic response of the thymocytes to various stimuli was assessed by blocking the flow of proliferating cells through their growth–division cycle at metaphase with colchicine (0.062 m*M*) and then determining the accumulation with time of metaphase cells in the population (5, 6).

Cyclic AMP and its physiologically active dibutyryl derivative (N^6-2′-*O*-dibutyryl-c-AMP) were obtained from Sigma (St. Louis) and Schwarz (Orangeburg, N. Y.), respectively. Normal AMP (adenosine 5′-monophosphate) was obtained from Sigma. These cyclic nucleotides were added to cell suspensions at the mitogenic optimally effective concentration of 10^{-7} *M* which is within the normal physiological range of intracellular concentrations (11, 12). Bovine growth hormone was obtained from Calbiochem (Los Angeles). The mitogenicity of growth hormone under these experimental conditions is due to hormonal action and not to a nonspecific nutritional effect (5).

Results and Discussion. After thymocyte populations were suspended in the simple, colchicine-containing glucose–salts medium, cells of the proliferating subpopulation [which occupied about 20% of the total cell population (5,13)] continued to flow into mitosis until the proportion of metaphase cells reached a maximum value of 4.3% by 4 hr (Fig. 1). When the medium contained 12 μg (0.01 USP units) of growth hormone/ml (the lowest maximally effective concentration), there was a large and prolonged increase in the flow of cells into mitosis which became significant after only 2 hr of incubation (Fig. 1).

An acceptable and commonly used method of determining whether this mitogenic action of growth hormone is mediated by cyclic AMP would be to show that methyl xanthines

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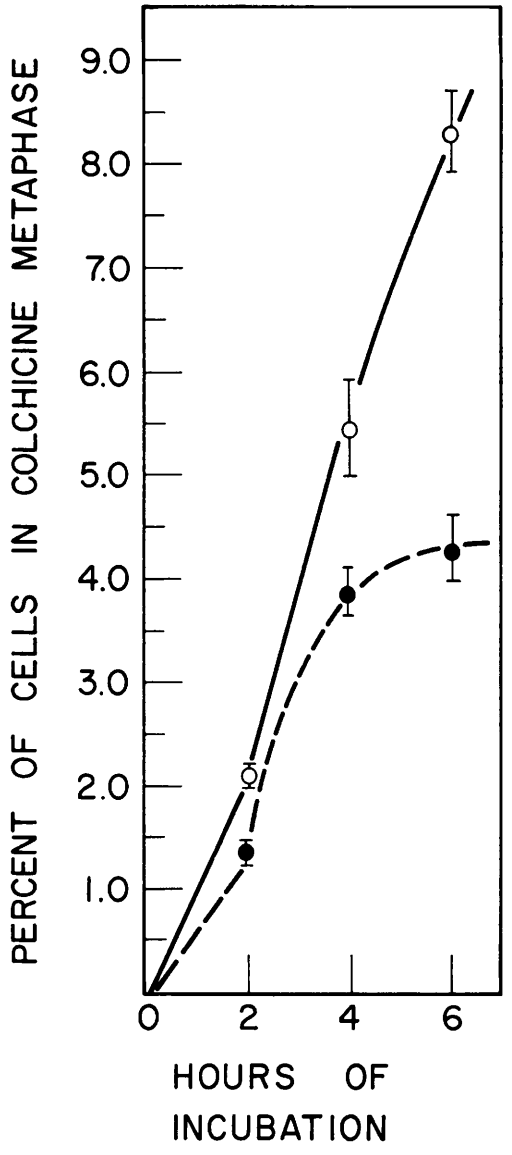

FIG. 1. The effect of growth hormone on the flow of thymocytes into mitosis: (●), cells incubated in colchicine-containing glucose–salts medium; (○), cells incubated in the same medium plus 12 μg of growth hormone/ml. Each point is the mean ± SEM of at least four determinations.

such as caffeine can increase the hormone's effectiveness (8, 11, 12). The methyl xanthines are known to specifically inhibit the action of the intracellular phosphodiesterase which converts cyclic AMP to the inactive adenosine 5′-monophosphate (8). Thus, these compounds allow a greater accumulation of cyclic AMP and thereby magnify the actions of those hormones which stimulate the formation of the cyclic nucleotide.

To implement this method, thymocyte populations were exposed to the highest concentration of growth hormone which did not affect mitotic activity (4 μg or 0.003 USP units/ml of medium) *per se* in the presence and absence of 0.4 m*M* caffeine which was also the highest concentration unable to affect mitotic activity (although higher concentrations were mitogenic). Figure 2 shows

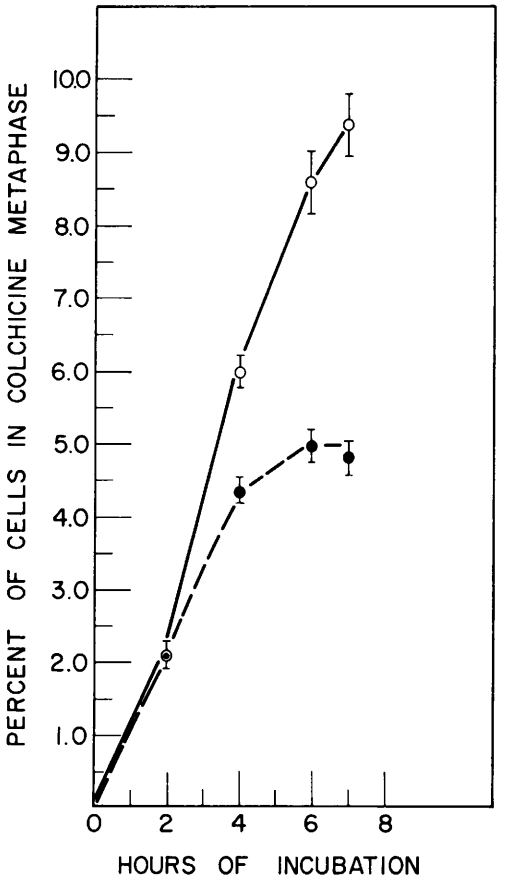

FIG. 2. The ability of caffeine to increase the mitotically stimulating action of growth hormone on thymocytes: (●), cells incubated in colchicine-containing glucose–salts medium having 4 μg of growth hormone/ml. (○), cells incubated in the same medium containing 4 μg of growth hormone/ml and 0.4 m*M* caffeine. Each point is the mean ± SEM of eight determinations.

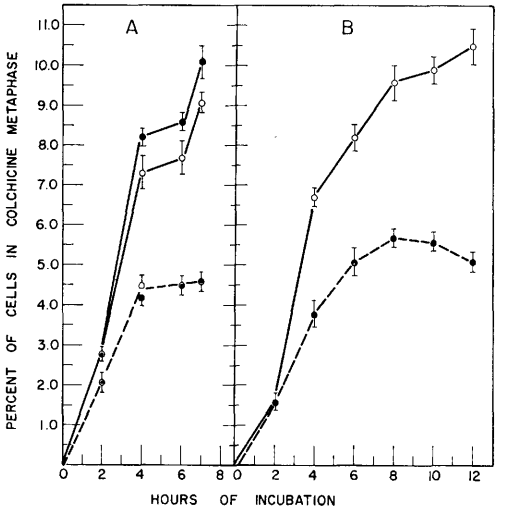

FIG. 3. The mitogenic action of cyclic AMP on rat thymocytes: (A) cells were incubated in glucose-salts medium; (B) cells were maintained in MAC-1 medium; (●---●), control; (○---○), the medium contained 10^{-7} *M* adenosine 5′-monophosphate; (●—●), the medium contained cyclic AMP; (○—○), the medium contained dibutyryl cyclic AMP. Each point is the mean ± SEM of at least four determinations.

that in the presence of the nonstimulatory level of caffeine this normally ineffective level of growth hormone was able to strongly and, in fact, maximally increase the flow of cells into mitosis; caffeine had therefore conferred upon the 4 μg of growth hormone/ml the stimulatory capacity of 12 μg/ml (compare Figs. 1 and 2).

If, as these observations clearly suggest, cyclic AMP is the mediator of growth hormone's mitogenic action, then cyclic AMP itself must be able to stimulate mitotic activity. This postulate was confirmed by the observations that, like growth hormone, cyclic AMP and its dibutyryl derivative strongly and rapidly (between 2 and 4 hr) promoted the flow of cells into metaphase while normal AMP was completely ineffective (Fig. 3A). This stimulatory action of the cyclic nucleotides was not due to some nonspecific correction of the nutritional inadequacy of the glucose–salts medium since these compounds also stimulated mitotic activity in cell populations suspended in the complex MAC-1 medium which, among other things, contains ATP, purines and pyrimidines (Fig. 3B).

We conclude that growth hormone's physiologically significant mitogenic action on lymphoid cells is mediated by cyclic AMP. Since this hormone's lipolytic action on adipose tissues is also mediated by the cyclic nucleotide (14, 15), it appears that growth hormone will be added to the growing list of hormones which use this compound as an intracellular "messenger" to implement their various actions. With respect to the actual mechanism of the hormone's mitogenicity, the available evidence suggests that growth hormone and its probable intracellular "messenger", cyclic AMP, act by stimulating the flow of cells into the S (or deoxyribonucleic acid-synthetic phase) of their growth–division cycle (5, 6, 16).

Summary. Bovine growth hormone stimulates the progression of rat thymocytes (maintained *in vitro*) into mitosis. The mitogenic capacity of growth hormone is considerably increased by caffeine which suggests that the hormonal action is mediated by adenosine 3′5′-monophosphate. In support of this suggestion, it is shown that cyclic AMP has the necessary ability to strongly stimulate mitotic activity in thymocyte populations.

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