CALCIUM-DEPENDENT STIMULATION OF PROLACTIN RELEASE IN RAT ANTERIOR PITUITARY *IN VITRO* BY N^6-MONOBUTYRYL ADENOSINE 3′,5′-MONOPHOSPHATE*

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1. Introduction

It is well accepted that adenosine 3′,5′-monophosphate (cyclic AMP), or its derivatives, stimulate the release of growth hormone [1–5], thyrotropic hormone [6–8], adrenocorticotropic hormone [9, 10], luteinizing hormone [11] and follicle-stimulating hormone [12]. However, no such evidence had yet been obtained for another anterior pituitary hormone, prolactin. In fact, while theophylline, a phosphodiesterase inhibitor, which presumably acts through increased intracellular levels of cyclic AMP, had been reported to stimulate prolactin release *in vitro* [13], no direct stimulation of hormonal release by exogenous derivatives of cyclic AMP could be detected [1, 2, 14].

Since it was thought, until recently, that the influence of the hypothalamus on prolactin secretion was exclusively inhibitory [15–21] through the action of the prolactin-inhibiting factor (PIF), while it was known that the hypothalamus had a stimulatory action on the secretion of the 5 other pituitary hormones, this apparent unresponsiveness of prolactin to cyclic AMP was ascribed to the postulated unique mode of control of the secretion of this hormone [1, 2]. However, the recent demonstration of prolactin-releasing activity in extracts of median eminence [22, 23] prompted a study of a possible stimulatory role of cyclic AMP on prolactin secretion, an effect already demonstrated for the 5 other anterior pituitary hormones [1–12].

The data presented in this paper show clearly that the N^6-monobutyryl derivative of cyclic AMP stimulates prolactin release from female rat anterior pituitary gland *in vitro* and that this stimulatory effect of the cyclic nucleotide is Ca^{2+}-dependent.

2. Materials and methods

Adult female Sprague-Dawley rats (obtained from Canadian Breeding Farms, St-Constant, Laprairie) weighing from 200–250 g were used throughout these experiments. Animals were kept in a sound proof room at 25 ± 1°, 50 to 55% humidity and on a 12 hr dark-light schedule for at least 1 week before use, during which they were allowed water and Purina Laboratory Chow ad libitum. Experiments were always begun between 7:30 and 9:00 a.m. to eliminate a possible diurnal variation of the secretory activity of the anterior pituitary gland. N^6-monobutyryl cyclic AMP was a commercial product of Boehringer Mannheim GmbH. Acrylamide and N,N′-methylenebisacrylamide were obtained from Eastman and recrystallized before use [24]. Preparation of hemipituitaries and incubation in Krebs-Ringer bicarbonate buffer [25] containing 11 mM D-glucose were performed essentially as described [3] and as specified in the legends to figures.

Growth hormone and prolactin account for approx. 95% of the proteins secreted into the incubation medium under our experimental conditions [3] and their release was measured after separation of the pro-

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teins of the incubation medium by polyacrylamide gel electrophoresis [26]. After the runs, the gels were stained with 0.005% amido black in 7.5% acetic acid and scanned at 635 nm with a Gilford spectrophotometer after removal of excess stain by a few changes of 7.5% acetic acid. Peaks corresponding to growth hormone and prolactin [3] were then copied on paper, cut out and weighted. The amounts of hormones released were then calculated from a standard curve using beef serum albumin as standard. The results are expressed as μg of hormone per ml of incubation medium.

3. Results

Fig. 1 shows clearly that the N^6-monobutyryl derivative of cyclic AMP stimulates markedly the release of both growth hormone and prolactin in rat anterior pituitary gland *in vitro*. During a 60 min incubation period, the amounts of growth hormone and prolactin released in the presence of the cyclic nucleotide are, respectively, of 200 and 150% over control. Similar results have been obtained using various N^6- and C8-derivatives of cyclic AMP (data not shown).

Fig. 2 shows the time course of both basal and cyclic AMP-induced release of GH and LTH in normal and modified (no Ca^{2+}, 12.5 mM Mg^{2+}) KRBG incubation medium. It can be seen (fig. 2A) that there is already a 50% increase of prolactin release 10 min after addition of mbcAMP to the incubation medium. The stimulation by mbcAMP increases at later time intervals and reaches 130% over control after 2 hr of incubation. The release of growth hormone under both basal and cyclic AMP-induced conditions (fig. 2B) shows time characteristics very similar to those of prolactin, although the absolute values are approx. twice for GH as compared to LTH.

Since it was known that calcium is required for the hypothalamic factor-induced release of luteinizing [27, 28], thyrotropic [29], and adrenocorticotropic [30] hormones, and for the dibutyryl cyclic AMP-induced release of adrenocorticotropic hormone [30], it was felt of interest to determine whether the cyclic AMP-induced release of prolactin shared the Ca^{2+}-dependence found for the other anterior pituitary hormones. It can be seen (fig. 2A) that in the absence of Ca^{2+} and presence of 12.5 mM Mg^{2+} in the incubation medium, the basal release of prolactin is 30%

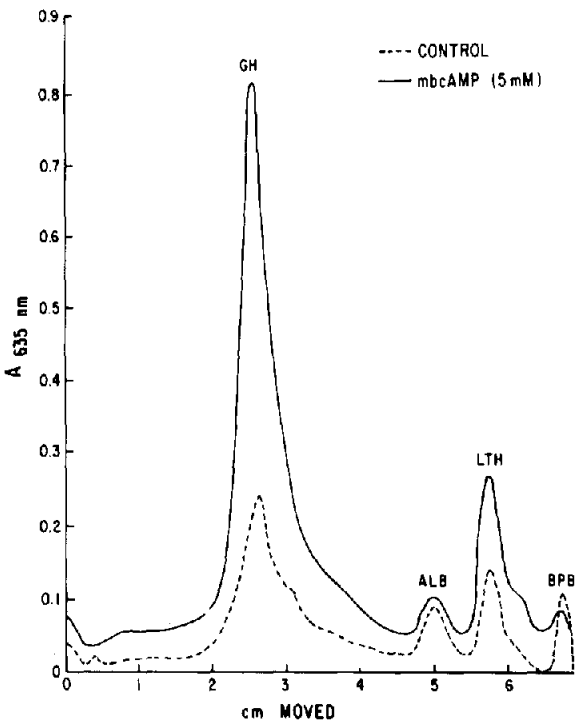

Fig. 1. Stimulation of growth hormone and prolactin release by 5 mM N^6-monobutyryl cyclic AMP (mbcAMP). Eight pituitary halves were incubated for 2½ hr at 37° in 2.5 ml of Krebs-Ringer bicarbonate buffer containing 11 mM D-glucose (KRBG) and 20 μCi of ^{3}H-leucine per ml as described [3]. Hemipituitaries were then transferred to KRBG alone or to KRBG containing 5 mM mbcAMP for a further 60 min incubation period. 250 μl aliquots of the incubation medium were lyophilised and run on polyacrylamide gels. The amounts of growth hormone and prolactin released were measured as described under "Materials and methods". GH, growth hormone; LTH, prolactin, Alb, albumin.

reduced during the first 10 min of incubation under both basal and cyclic-AMP induced conditions. It is also quite clear that the dependence upon Ca^{2+} increases with time, a 90% inhibition of basal release of prolactin being observed between 60 and 120 min of incubation. In modified medium, the stimulation of prolactin release normally observed with mbcAMP is 75% reduced at 60 min and is virtually abolished at later time intervals. Comparable effects of absence of Ca^{2+} and increased Mg^{2+} concentration are observed on growth hormone release, although the data suggest that the Ca^{2+}-dependence of GH release is of somewhat lower magnitude (fig. 2B).

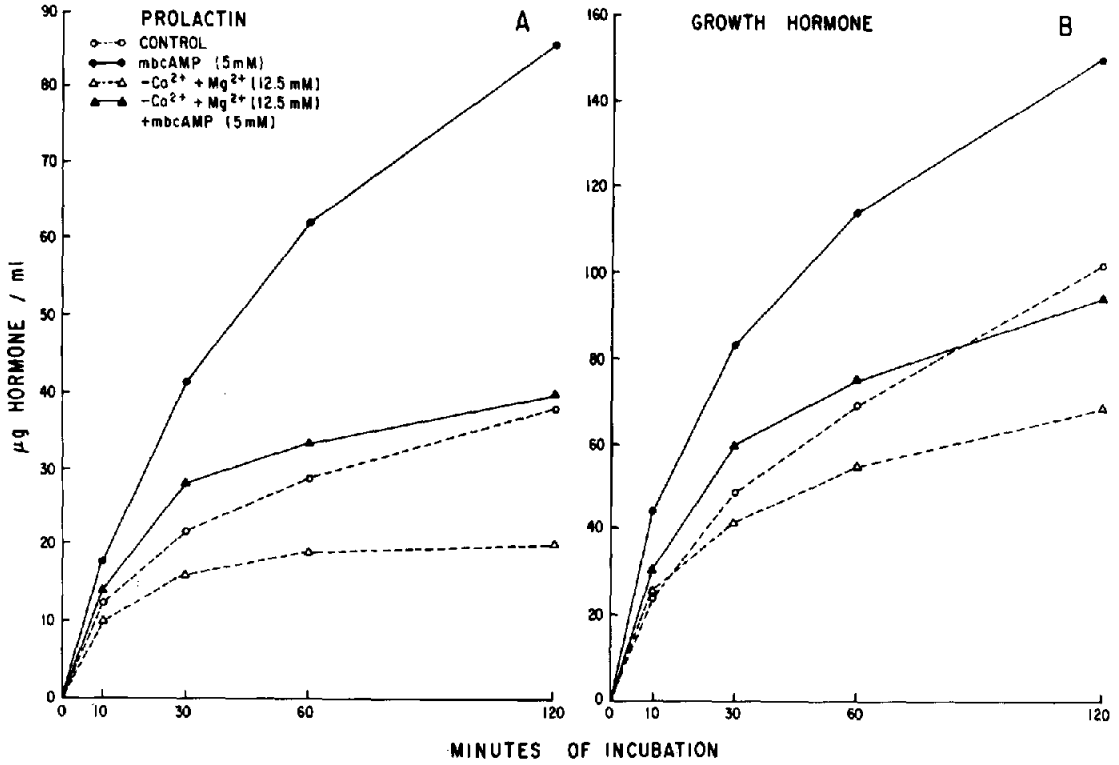

Fig. 2. Time course of release of growth hormone and prolactin in the presence or absence of 5 mM N^6-monobutyryl cyclic AMP in normal or low Ca^+-high Mg^{2+} KRBG medium. Twelve pituitary halves were incubated for 2½ hr at 37° in 1 ml of KBRG containing 20 µCi ^{3}H-leucine. Pituitaries were then transferred into KRBG or KRBG containing no Ca^{2+} and 12.5 mM Mg^{2+}, in the presence or absence of 5 mM mbcAMP. At the indicated times, the incubation medium was changed and appropriate aliquots were taken for measurement of hormonal release (panel A, prolactin; panel B, growth hormone). o- - - -o, control in normal KRBG; •- - - -•, 5 mM mbcAMP in normal KRBG; △- - - -△, modified KRBG, no Ca^{2+} and 12.5 mM Mg^{2+}; ▲- - - -▲, 5 mM mbcAMP in modified KRBG.

4. Discussion

The action of many polypeptide hormones is at least partly mediated by the adenyl cyclase system [31]. Recent data indicate that cyclic AMP is also involved in the action of the hypothalamic releasing hormones in the anterior pituitary gland [1–14, 32–39]. It has in fact been found that hypothalamic extracts stimulate adenohypophyseal adenyl cyclase activity [32], that cyclic AMP-dependent protein kinase is widely distributed among subcellular structures of the anterior pituitary gland [34–36], and that cyclic AMP stimulates both total protein synthesis [3, 37] and the release of specific hormones by the adenohypophysis [1–14, 38].

Although the demonstration of a theophylline-induced secretion of prolactin [13] was suggestive of a role of cyclic AMP in the release of this hormone, conclusive data for a stimulatory role of cyclic AMP on prolactin secretion had so far been lacking. Fig. 1 and 2 show clearly that the N^6-monobutyryl derivative of cyclic AMP enhances markedly the release of prolactin in rat anterior pituitary gland *in vitro*. As measured after separation by polyacrylamide gel electrophoresis, the stimulation of prolactin release by mbcAMP is already 50% over control at 10 min of incubation and reaches 150% over the control rate at 60 min. Many N^6- and C8 derivatives of cyclic AMP lead to similar increases of prolactin release *in vitro* (data not shown). These findings of a marked and

rapid release of both prolactin and growth hormone under the influence of cyclic AMP derivatives are supported by ultrastructural studies which show a marked increase of the exocytosis processes in the prolactin-secreting cells 5 min after addition of dibutyryl cyclic AMP and an almost complete depletion of intracellular prolactin-containing secretory granules after 2 hr of incubation in presence of the cyclic nucleotide [38].

Moreover, our data confirm that Ca^{2+} is required for the basal release of prolactin [13] and show clearly that the mbcAMP-induced release of prolactin is Ca^{2+}-dependent, a characteristic shared by growth hormone (fig. 2B) and adrenocorticotropic hormone [30].

The recent findings of prolactin releasing activity in rat [22] and porcine [23] hypothalamus, and the present demonstration of a Ca^{2+}-dependent stimulation of prolactin release by N^6-monobutyryl cyclic AMP in rat anterior pituitary *in vitro* indicate that the control of prolactin secretion may, after all, share many characteristics with the other pituitary hormones, and suggest a role of the adenyl cyclase system in the control of activity of the prolactin-secreting cells.

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