PITUITARY CYCLIC AMP PRODUCTION AND MECHANISM OF LUTEINIZING HORMONE RELEASE

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1. Introduction

Secretion of anterior pituitary luteinizing hormone (LH) is controlled by the hypothalamic luteinizing hormone–releasing hormone (LH–RH) which has recently been isolated [1,2]. Crude hypothalamic extract containing LH-releasing activity was reported to stimulate adenylate cyclase and to increase the cyclic AMP level in the pituitary gland [3]. Recently it was found that synthetic LH–RH increased adenylate cyclase activity [4] cyclic AMP level and concomitantly stimulated LH release [5–8]. Hence it was suggested that cyclic AMP is the mediator of LH–RH action on LH release [5–7]. This conclusion was further supported by the findings that analogues of LH–RH which increased cyclic AMP production also caused LH release [9]. The addition of dibutyryl cyclic AMP (DBC) to the incubation medium was reported to enhance the release of LH [7,10], while other workers found no increase of LH upon the addition of this drug [11,12]. The present investigation was intended to clarify whether the increase in pituitary cyclic AMP production by LH–RH is an obligatory step in LH release.

2. Materials and methods

Two-month-old Wistar-derived male rats of the departmental colony were housed in air-conditioned quarters, illuminated between the hours 05:00 and 19:00. Pelleted food (Ralston Purina Co.) and water were offered without restriction.

LH–RH (synthetic decapeptide) was a generous gift of Dr N. Yanaihara. Prostaglandin E_2 (PGE_2) was generously made available by Dr J. Pike of the Upjohn Co., Kalamazoo, Michigan. Flufenamic acid (*N*-(α-α-α-trifluoro-*m*-tolyl) anthranilic acid), a gift of Rafa Laboratories, Jerusalem, was dissolved in 0.1 N NaOH (10 mg/ml). Theophylline (1,3-dimethylxanthine) was purchased from Sigma Chemical Corp., St. Louis, Missouri; IBMX (3-isobutyl-1-methylxanthine) from Aldrich Chemical Co., Milwaukee, Wisconsin and dibutyryl cyclic AMP from Boehringer and Söhne, Mannheim. The purified cholera toxin (choleragen) used in this study (Lot 1071) was prepared under contract for the National Institute of Allergy and Infectious Diseases (NIAID) by R. A. Finkelstein, Ph.D., The University of Texas, Southwestern Medical School, Dallas, Texas [13].

For the in vitro studies, anterior pituitaries were cut in half. Two hemipituitaries (derived from different animals), were placed in each flask and initially incubated at 37°C for 2 h in 1 ml of Krebs-Ringer bicarbonate (KRB) pH 7.4, containing glucose (1 mg/ml). The medium was changed to 1 ml of KRB containing various drugs or hormones as indicated in the text, and the incubation was continued for another 4 h. At the end of the incubation period, aliquots of the medium were analyzed for LH content by radioimmunoassay [14] and the slices were placed immediately in tubes containing hot Na-acetate buffer pH 4.0. The content of cyclic AMP was determined by a competitive protein binding assay [15].

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3. Results

Incubation of hemipituitaries for 4 h with LH–RH, PGE_2, choleragen, theophylline or IBMX, raised pituitary cyclic AMP accumulation by a factor of 4, 13, 27, 5, and 5, respectively (fig.1). However, only LH–RH stimulated LH release into the medium (3-fold increase; fig.1). Dibutyryl cyclic AMP (1 mM; fig.1) and 1 mM 8-bromo-cyclic AMP (data no shown) were without effect on LH release.

Flufenamic acid (3-100 μg/ml) did not influence pituitary cyclic AMP accumulation; however at 10 μg/ml or higher concentration the drug abolished LH–RH stimulation of cyclic AMP production. Nevertheless, flufenamic acid (10 μg/ml) did not inhibit LH–RH stimulation of LH release into the medium (fig.2). At high concentration (100 μg/ml), the drug on its own caused LH release (70%).

4. Discussion

Cyclic AMP is implicated in the mechanism of action of many protein and peptide hormones. Since the pituitary adenylate cyclase-cyclic AMP system was recently shown to be stimulated by LH–RH in vitro [5–8], it was suggested that cyclic AMP is an essential mediator in the mechanism of LH–RH action on LH release [5–7]. However, under various experimental conditions we were able to dissociate

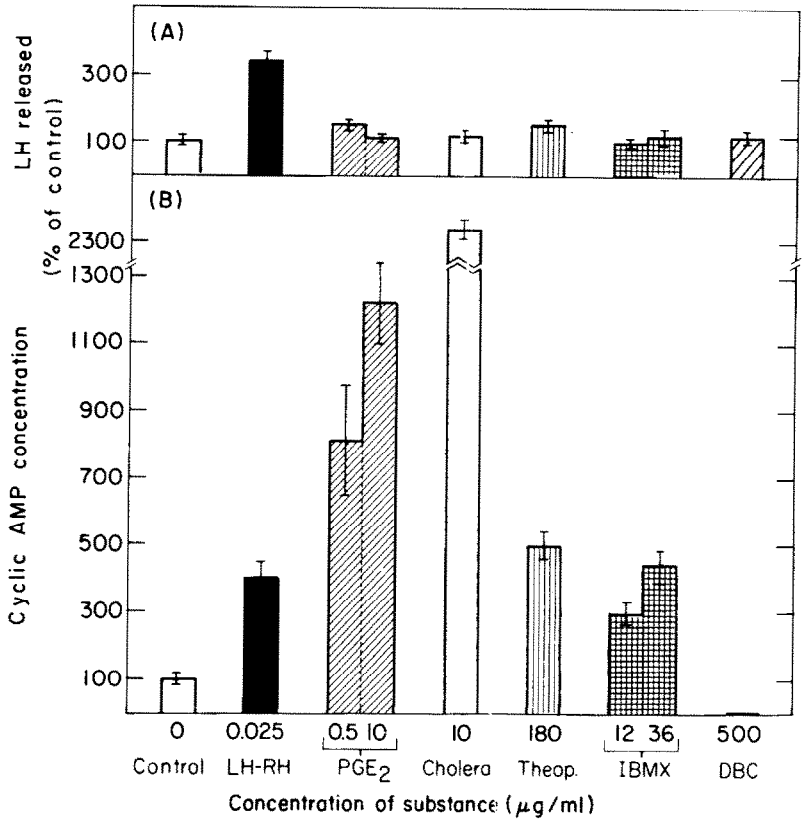

Fig.1. In vitro effects of LH–RH, PGE_2, choleragen (cholera), theophylline (theop), IBMX and dibutyryl cyclic AMP (DBC) on LH release (A) and cyclic AMP level in the pituitary (B). Bisected anterior pituitaries were incubated for 2 h in KRB. The medium was then changed to KRB containing the drugs indicated, and the incubation was continued for another 4 h. At the end of the incubation period, aliquots of the medium were analyzed for LH content by radioimmunoassay and pituitaries were taken to cyclic AMP determination as described in Materials and methods. Basal LH release to the medium was 18.5 ± 2.0 μg/ml, and basal cyclic AMP level was 0.44 ± 0.04 pmol/mg tissue wet weight. Vertical brackets indicate S. E. M. for 10 determinations.

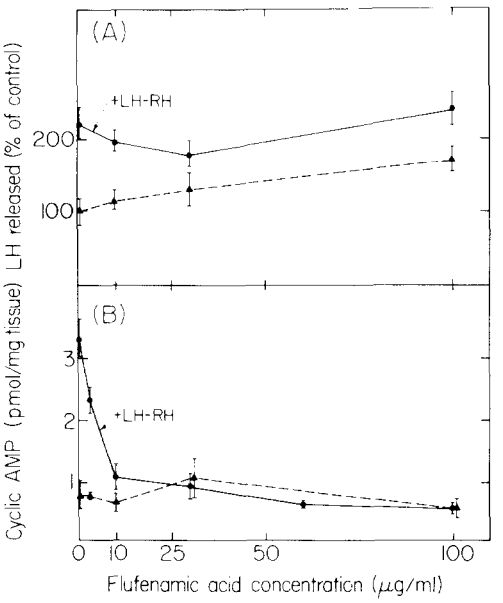

Fig.2. Effect of flufenamic acid on the stimulatory action of LH–RH on LH release (A) and cyclic AMP accumulation (B) by the pituitary in vitro. Anterior pituitaries were incubated for 2 h in KRB. The medium was then changed and various doses of flufenamic acid (0–100 μg/ml) alone (▲--▲), or together with 25 ng/ml of LH–RH (●——●), were added. The incubation was continued for another 4 h. At the end of the incubation period aliquots of the medium were analyzed for LH content and the pituitaries for cyclic AMP content as described above. Basal LH released to the medium was 28.5 ± 4 μg/ml. Vertical brackets indicate S. E. M. for 10 determinations.

between the action of LH–RH on cyclic AMP production and on LH release.

While there is a general agreement that PGE increases pituitary cyclic AMP production [3,4,7,16], reports differ as to the effect of PGE on LH release. Makino [7] and Ratner et al. [16] found increased LH release when PGE was added to incubated pituitaries, but other workers failed to find any increase in LH release after short [17] or long incubation periods with PGE ([18] and present results fig.1). The disagreement concerning the PGE effect on LH release might be explained by strain differences between the animals used, or by the use of different types of prostaglandins (PGE_2 versus PGE_1). A similar dissociation between cyclic AMP increase and LH release was observed when another stimulator of adenylate cyclase, namely choleragen, was incubated with pituitaries (fig.1). Since the gonadotrophs constitute only 5–10% of the pituitary cell population, it could be argued that PGE_2 and choleragen affect pituitary cell types other than the gonadotrophs and hence had no effect on LH release. However, it seems unlikely that these non-specific drugs differentiate between the gonadotrophs and other pituitary cell types. Furthermore, incubation of pituitaries with theophylline (1 mM) or relatively small amounts of IBMX (0.06 mM) increase pituitary cyclic AMP accumulation without affecting LH release (fig.1). These drugs probably inhibit phosphodiesterase activity in all the different cell types. Thus it seems to us that the effect is exerted on all the different cell types, and that the increase in cyclic AMP is not relevant to stimulation of LH release. Addition of cyclic AMP derivatives like dibutyryl cyclic AMP (DBC) and 8-bromo-cyclic AMP to the incubation medium did not stimulate LH release ([11,12] and present results, fig.1), which strengthens this conclusion. It might be argued that these cyclic AMP derivatives do not penetrate the gonadotrophs. However, Makino [7] found stimulation of LH release upon the addition of DBC to incubated pituitaries. Isolated gonadotrophs might serve as a suitable tool for further investigation concerning this problem.

In recent studies (data not shown) we observed a difference in responsiveness to LH–RH between male and female rats. While in the male we could always observe an increase in cyclic AMP and LH release, in response to LH–RH in vitro, in female rats no increase in cyclic AMP was observed in some experiments, although LH release was stimulated.

In a different set of conditions it was possible to demonstrate a stimulatory action of LH–RH on pituitary LH release without an effect on cyclic AMP production: flufenamic acid (10 μg/ml) abolished LH–RH stimulation of cyclic AMP production but did not affect its action on LH release (fig.2).

The possibility that elevation of cyclic AMP by LH–RH or its analogues [6,9] may mediate the effect of LH–RH on LH biosynthesis is not excluded by the present experiments. However, the present results and recent studies [17,19,20] suggest that the stimulation of pituitary cyclic AMP formation by LH–RH is not an obligatory step in the mechanism of LH release.

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References

[1] Burgus, R., Butcher, M., Ling, N., Monham, M., Rivier, J., Fellows, R., Amoss, M., Blackwell, R., Vale, W. and Guillemin, R. (1971) C. R. Acad. Sci., Paris, 273, 1611–1613.
[2] Matsuo, H., Baba, Y., Nair, R. M. G., Arimura, A. and Schally, A. V. (1971) Biochem. Biophys. Res. Commun. 43, 1334–1337.
[3] Zor, U., Kaneko, T., Schneider, H. P. G., McCann, S. M., Lowe, P. I., Bloom, G., Borland, B. and Field, J. B. (1969) Proc. Natl. Acad. Sci. USA. 63, 918–925.
[4] Deery, D. J. and Howell, S. L. (1973) Biochim. Biophys. Acta. 329, 17–22.
[5] Borgeat, P., Chavancy, G., Dupont, A., Labrie, F., Arimura, A. and Schally, A. V. (1972) Proc. Natl. Acad. Sci. USA. 69, 2677–2681.
[6] Kaneko, T., Saito, S., Oka, H., Oda, T. and Yanaihara, N. (1973) Metabolism 22, 77–80.
[7] Makino, T. (1973) Am. J. Obst. Gynecol. 115, 606–613.
[8] Naor, Z., Koch, Y., Bauminger, S. and Zor, U. (1975) Prostaglandins 9, 211–221.
[9] Borgeat, P., Labrie, F., Cote, J., Ruel, F., Schally, A. V., Coy, D. H., Coy, E. J. and Yanaihara, N. (1974) Mol. Cell. Endocrinol. 1, 7–20.
[10] Groom, G. V. and Boyns, A. R. (1973) J. Endocrinol. 59, 511–522.
[11] Wakabayashi, K., Date, Y. and Tamaoki, B. (1973) Endocrinology 92, 698–704.
[12] Tang, L. K. L. and Spies, H. G. (1974) Endocrinology 94, 1016–1021.
[13] Finkelstein, R. A. and LoSpalluto, J. J. (1970) J. Infect. Dis. Suppl. 121, p. S63.
[14] Daane, T. A. and Parlow, A. F. (1971) Endocrinology 88, 653–663.
[15] Gilman, A. G. (1970) Proc. Natl. Acad. Sci. USA. 67, 305–312.
[16] Ratner, A., Wilson, M. C., Srivastava, L. and Peake, G. T. (1974) Prostaglandins 5, 165–171.
[17] Zor, U., Lamprecht, S. A., Kaneko, T., Schneider, H. P. G., McCann, S. M., Field, J. B., Tsafriri, A. and Lindner, H. R. (1972) In: Advances in Cyclic Nucleotide Research (Greengard, P., Paoletti, R. and Robison, G. A., eds), Vol. 1, pp. 503–520, Raven Press, New York.
[18] Chosieng, P., Naor, Z., Koch, Y., Zor, U. and Lindner, H. R. (1975) Neuroendocrinology 17, 12–17.
[19] Rigler, G. L., Ratner, A., Srivastava, L. and Peake, G. T. (1975) Endocrine Society 57th Annual Meeting, 102, New York.
[20] Zor, U., Chayoth, R., Kaneko, T., Schneider, H. P. G., McCann, S. M. and Field, J. B. (1974) Metabolism 23, 549–559.