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Effects of Hypothalamic-Releasing Hormones on LH, FSH, and Prolactin in Pituitary Monolayer Cultures[1] (39171)

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Various substances from the hypothalamus have been demonstrated to affect the secretion of adenohypophyseal hormones (1). Evidence has also been presented to suggest the involvement of adenosine 3′:5′-cyclic monophosphate (cAMP) and its generating system (adenylate cyclase) in the action of several hypothalamic-releasing hormones (2).

This report concerns the direct effect of hypothalamic extract (HE) and synthetic releasing hormones in monolayer cultures of pituitary cells. The effectiveness of adenosine cyclic nucleotide in inducing the release of LH, FSH, and prolactin (PRL) is also reported.

Materials and methods. Anterior pituitaries of male Sprague–Dawley rats (200 to 250 g) were used throughout the study. Materials for preparing the tissue cultures were purchased from the Grand Island Biological Co. Synthetic LH-RH (Lot 19-192, Abbott Labs.) and rat hypothalamic extract (NIAMD-RatHE-RP-1) were graciously provided by the Hormone Distribution Office, NIAMD, NIH, and somatostatin (GH-RIH, the linear form) was generously provided by Dr. R. Guillemin. Synthetic TRH was purchased from Calbiochem; N^6,$O^{2'}$-dibutyryl adenosine 3′:5′-cyclic monophosphate (DBcAMP), cAMP, adenosine, and theophylline were purchased from Sigma Chemical Co.

Pituitary cultures. Methods for preparing the monolayer cell cultures have been described previously (3), but in this study male rat sera were substituted for monkey sera in the growth media. Four- or five-day-old cultures were washed twice with 3 ml of Dulbecco's modified Eagle's medium (DMEM) without sera. Test compounds were then added to the cultures in 25 ml of DMEM and incubated at 37° for 4 hr. At the end of the incubation, the medium was collected and centrifuged at 2700*g* for 30 min at 6°. The supernatant was rapidly frozen and stored at −20° for later determinations of LH, FSH, and PRL with double antibody radioimmunoassay systems.[2] All data are presented as means + standard error of means (mean + SE). Significant differences between groups were determined by Duncan's new multiple range test (4).

Results and discussion. The data in Fig. 1 indicate that rat hypothalamic extract (RHE) significantly ($P < 0.05$) stimulates the release of LH, FSH, and PRL by these cultures in a dose-related fashion ($r = 0.99$, 0.95, and 0.89, respectively). However, the FSH response to RHE stimulation was less pronounced than the LH response. Although both the PRL release-inhibiting hormone (PRIH) and the PRL-releasing hormone (PRH) have been purified from bovine posterior pituitary-stalk median eminence (5), the fact that NIAMD-RatHE-RP-1 stimulated the release of PRL suggests that PRH activities predominate in this preparation. With different methods of preparation, a hypothalamic extract may exhibit predominantly PRIH (6) or PRH activities (7).

The direct effect of synthetic LH-RH on the cultures is shown in Fig. 2. After a 4-hr incubation, LH-RH at concentrations of 10^{-9} *M* or greater significantly ($P < 0.05$) stimulated LH and FSH release into the media, whereas up to 10^{-7} *M* LH-RH neither stimulated nor inhibited PRL release

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[2] LH-RIA: LH antiserum and purified ovine LH (LER-1056-C2) for iodination was supplied by Drs. G. D. Niswender and L. E. Reichert, Jr., respectively; NIAMD-LH-RP-1 was the reference standard. NIAMD-radioimmunoassay kits for FSH and PRL were supplied through the hormone distribution program, NIH.

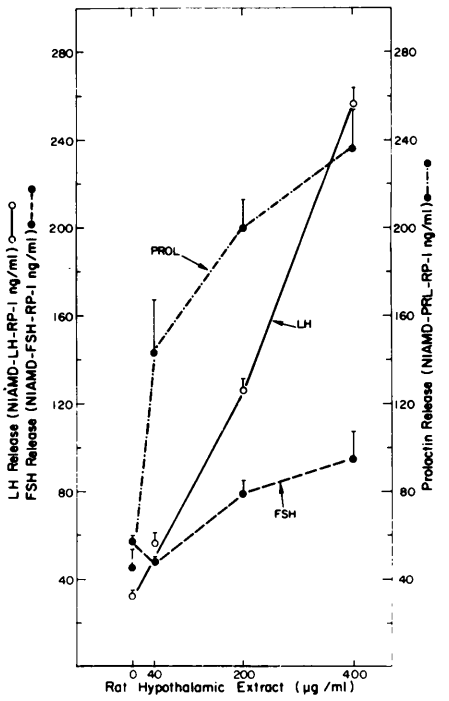

FIG. 1. Effect of various concentrations of RHE on LH, FSH, and PRL release. Day 5 cultures were incubated with RHE at 0, 40, 200, or 400 µg/ml for 4 hr at 37°. LH, FSH, and PRL concentrations in the media are shown as mean + SE of four replicate dishes.

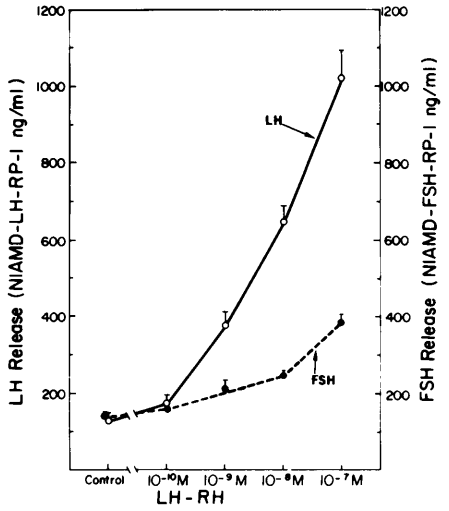

FIG. 2. Effect of various concentrations of LH-RH on LH and FSH release. Day 4 cultures were incubated with LH-RH at 0, 10^{-10}, 10^{-9}, 10^{-8}, or 10^{-7} *M* for 4 hr at 37°. LH and FSH concentrations in the media are shown as mean + SE of four replicate dishes.

(data not shown). Similar lack of effect of LH-RH on PRL secretion was previously reported by Blackwell *et al.* (8). A significant dose-related response to LH-RH was observed for LH and FSH (r = 0.99 and 0.94, respectively) although the magnitude of FSH reponse was again (see Fig. 1) less than that of LH. Moreover, with 10^{-8} *M* LH-RH, a significant ($P < 0.05$) rise in LH release into the media was detected by 30 min, whereas a rise in FSH release was not evident until 240 min (data not published). Whether these differences in LH and FSH responses are due to differences in the secretory processes for transferring the two hormones from inside to outside the cell or to differences in the two cell surface receptors for recognizing the releasing factor is not yet known. Evidence has been presented to suggest the existence of two specific pituitary binding sites for LH-RH which mediate the release of LH and FSH, respectively (9).

The direct effects of the two other synthetic hypothalamic hormones, TRH and GH-RIH, are shown in Fig. 3. TRH stimulated PRL release without any effect on LH or FSH release. These results confirm earlier findings in transformed and nontransformed cells of pituitaries (10–11). At 10^{-8} *M* concentration, GH-RIH did not affect the release of the three hormones; however, with higher concentrations, GH-RIH inhibition on PRL release was reported by Vale *et al.* (12).

The effects of adenine nucleotides and theophylline on the release of LH, FSH, and PRL are shown in Fig. 4. After a 4-hr incubation with 10^{-2} *M* of adenosine, cAMP, DBcAMP, or theophylline, LH and FSH released into the media was not significantly different from that of the control groups. This agrees with earlier findings in hemisected rat pituitaries (6, 13). However, in the cultures treated with DBcAMP or theophylline, PRL release was stimulated to 167 or 137% of the control level, respectively ($P < 0.05$). These results suggest that cAMP and the adenylate cyclase system are involved in the mechanism of PRL release. Their involvement in the LH and FSH release under identical conditions, however, was not evident and requires fur-

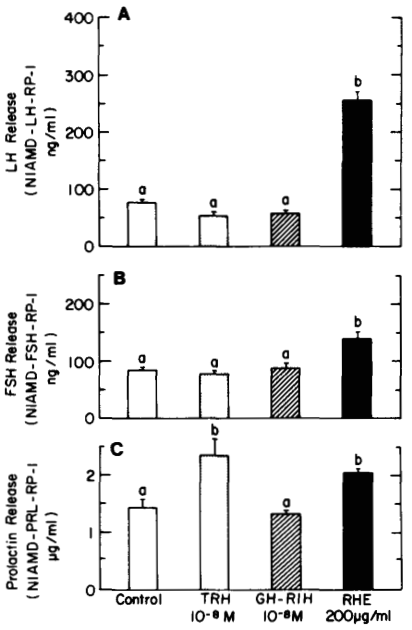

FIG. 3. Effect of TRH, somatostatin (GH-RIH), and RHE on the release of LH, FSH, and PRL. Day 4 cultures were incubated with 10^{-8} *M* TRH, 10^{-8} *M* GH-RIH, or 200 µg RHE/ml for 4 hr at 37°. (A) LH, (B) FSH, and (C) PRL concentrations in the media are shown as mean + SE of four replicate dishes. (Groups sharing the same alphabetical letter are not different from each other, $P > 0.05$).

ther studies, particularly in light of the findings that indicate the dissociation between activation of adenylate cyclase–cyclic AMP system and LH release in pituitary incubations (14).

Summary. Four-day-old pituitary monolayer cultures were incubated with various hypothalamic releasing hormones. Rat hypothalamic extract stimulated the release of LH, FSH, and PRL by these cultures in a dose-related fashion. Synthetic LH-RH stimulated the release of LH and FSH but not of PRL. Synthetic TRH increased the release of PRL but had no effect on LH or FSH. At 10^{-8} *M*, somatostatin did not affect any of the three adenohypophyseal hormones. Incubation with DBcAMP or theophylline also stimulated PRL release without any detectable effect on LH and FSH release. These data suggest the involvement of cyclic AMP–adenylate cyclase system in the mechanism of PRL release, but their involvement in gonadotropin release requires further studies.

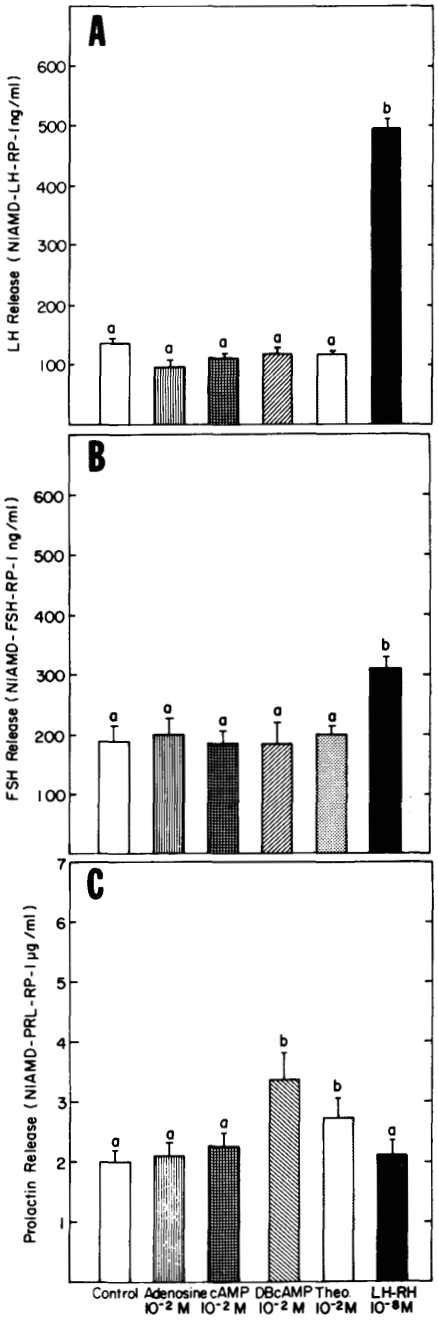

FIG. 4. Effect of adenine nucleotides and theophylline on the release of LH, FSH, and PRL. Day 5 cultures were incubated with DMEM alone, DMEM plus 10^{-2} *M* adenosine, 10^{-2} *M* cAMP, 10^{-2} *M* DBcAMP, 10^{-2} *M* theophylline, or 10^{-8} *M* LH-RH for 4 hr at 37°. (A) LH, (B) FSH, and (C) PRL concentrations in the media are shown as mean + SE of four replicate dishes. (Groups sharing the same alphabetical letter are not different from each other, $P > 0.05$).

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1. Schally, A. V., Arimura, A., Kastin, A. J., Science **179,** 341 (1973).
2. Robison, G. A., Butcher, R. W., and Sutherland, E. W., "Cyclic AMP," p. 388. Academic Press, New York (1971).
3. Tang, L. K. L., and Spies, H. G., Endocrinology **94,** 1016 (1974).
4. Steel, R. G. D., and Torrie, J. H., "Principles and Procedures of Statistics." McGraw-Hill, New York (1960).
5. Dular, R., LaBella, F., Vivian, S., and Eddie, L., Endocrinology **94,** 563 (1974).
6. Wakabayashi, K., Date, Y., and Tamaoki, B., Endocrinology **92,** 698 (1973).
7. Rivier, C., and Vale, W., Endocrinology **95,** 978 (1974).
8. Blackwell, R., Vale, W., Amoss, M., Burgus, R., Monahan, M., Rivier, J., Ling, N., and Guillemin, R., Amer. J. Physiology **224,** 176 (1973).
9. Spona, J., FEBS Letters **35,** 59 (1973).
10. Tashjian, A. H., Jr., Barowsky, N. J., and Jensen, D. K., Biochem. Biophys. Res. Commun. **43,** 516 (1971).
11. Vale, W., Blackwell, R., Grant, G., and Guillemin, R., Endocrinology **93,** 26 (1973).
12. Vale, W., Rivier, C., Brazeau, P., and Guillemin, R., Endocrinology **95,** 968 (1974).
13. Zor, U., Kaneko, T., Schneider, H. P. G., McCann, S. M., and Field, J. B., J. Biol. Chem. **245,** 2883 (1970).
14. Zor, U., Chayoth, R., Kaneko, T., Schneider, H. P. G., McCann, S. M., and Field, J. B., Metabolism **23,** 549 (1974).

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