tivities, volcanism, or emissions from vegetation.

Enrichments for Mg are near unity for both air and snow, with greater values for Pb and Cu. However, the EF_{air} for Pb and Cu are not as large as the values observed in more industrialized areas. For example, Rahn (*13*) reported mean values for EF_{air} of 3800 for Pb and 149 for Cu for 29 urban areas throughout the world. The unusually small aerosol enrichments for these two elements in our measurements reflect the nature of aerosols generated by combustion of wood and dung, with some influence of soil dust (*14*).

The very small enrichments for fresh snow at Kumjung imply a more crustal character for snow than for air. This is possibly due to a gradient in trace element concentrations in the valley: snow formed aloft would be more influenced by natural soil dust from the 6000-m peaks surrounding Kumjung, whereas air near the ground should be more influenced by the fires. Aerosol measurements in other parts of Nepal have shown evidence of combustion-generated material at ground level, with sulfates and soil dust found at higher elevations (*15*). The high atmospheric turbidity occasionally observed near the ground during these experiments also suggests the existence of a concentration gradient. Another possible explanation for the difference in enrichments is that crustal material, found in larger particle sizes than enriched aerosol, may be more efficiently scavenged as condensation nuclei during snowflake formation (*16*).

Dry deposition velocities were calculated as the ratio of the flux (in nanograms per square centimeter per second) to the airborne concentration (in nanograms per cubic centimeter), with the fluxes determined by taking the difference between old and fresh snow concentrations. Earlier work has shown the utility of this method, provided that sublimation is minimal and below-freezing temperatures are maintained (*6*, *17*). Such conditions prevailed during this study.

The greater values of deposition velocity for Al and Mg reflect larger particle sizes. This conclusion is supported by the impactor data, which indicate a mass mean aerodynamic diameter of 9 μm for Mg and 0.9 μm for Pb (*18*). Smaller deposition velocities and particle sizes for enriched relative to crustal elements have been reported for other remote and populated areas (*19*).

Overall, these data for Pb, Cu, Al, Mg, and C suggest that local fires fueled with wood and dung significantly influence air quality in a populated Himalayan valley. Differences in the chemical composition of freshly fallen snow as compared with that of air in the valley indicate the pristine nature of air aloft.

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Gonadotropin-Releasing Hormone: Regulation of Adenosine 3′,5′-Monophosphate in Ovarian Granulosa Cells

Abstract. *The antigonadal effects of gonadotropin-releasing hormone in ovarian granulosa cells are due to attenuation of the adenosine 3′,5′-monophosphate (cyclic AMP) response to follicle-stimulating hormone. Agonists of gonadotropin-releasing hormone progressively inhibit adenylate cyclase and stimulate phosphodiesterase activities in cultured granulosa cells, indicating that blockade of gonadotropin action is attributable to the combined effects of decreased production and increased degradation of cyclic AMP.*

The induction of ovarian follicular differentiation by follicle-stimulating hormone (FSH) is mediated by adenosine 3′,5′-monphosphate (cyclic AMP), which elicits the expression of peptide hormone receptors, steroidogenic enzymes, and morphological maturation of granulosa cells (*1*). These responses are prevented by gonadotropin-releasing hormone (GnRH) and its potent agonist analogs, which inhibit developmental processes in the rat ovary by a direct action on granulosa cells in intact animals and in vitro (*1*, *2*). The detection of specific binding sites for GnRH in ovarian tissue indicates that this peptide expresses its inhibitory actions by way of receptor-dependent mechanisms (*3*). We recently demonstrated that GnRH suppresses FSH-induced accumulation of both intracellular and extracellular cyclic AMP and guanosine 3′,5′-monophosphate (cyclic GMP) in granulosa cells isolated from hypophysectomized rats

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with implanted diethylstilbestrol (*4*). Such actions of GnRH on cyclic AMP production have not been correlated with short-term effects of the peptide on adenylate cyclase activity, either in follicular or luteal tissue (*5*, *6*). We now present evidence that an analog of GnRH stimulates phosphodiesterase (E.C. 3.1.4.17) activity in FSH-treated granulosa cells, and that inhibition of phosphodiesterase prevents the GnRH-induced reduction of cyclic AMP accumulation. We have also observed a progressive inhibitory effect of the GnRH agonist on adenylate cyclase (E.C. 4.6.1.1) activity during culture with granulosa cells. These data suggest that GnRH agonists exert inhibitory effects on ovarian function via decreased synthesis and increased degradation of cyclic AMP, with consequent reduction of cyclic nucleotide concentrations in gonadotropin-stimulated ovarian cells.

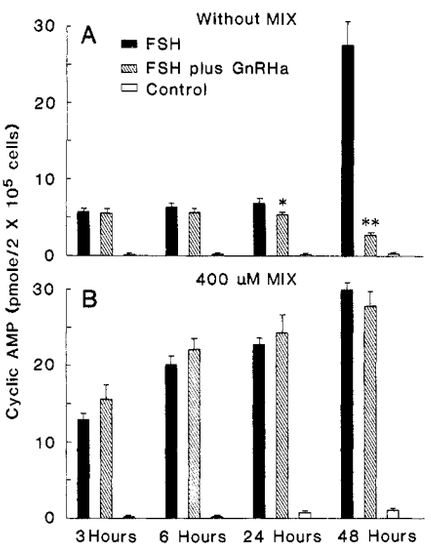

Fig. 1. Effect of a phosphodiesterase inhibitor on cyclic AMP production by granulosa cells cultured with FSH and GnRHa. Female hypophysectomized rats (25 days old; Hormone Assay Laboratories) received diethylstilbestrol in implanted Silastic capsules (10 mm) at the time of hypophysectomy and were killed 5 days later. After removal of the ovaries, granulosa cells were isolated and cultured (2×10^5 cells per milliliter) in McCoy's 5A medium (modified, without serum) supplemented with 10 m*M* Hepes buffer, *p*H 7.4, 4 m*M* L-glutamine, penicillin (100 U/ml), and streptomycin sulfate (100 μg/ml) (*4*). Ovine FSH (NIH-FSH-S7, 5 μg, or NIH-FSH-S13, 250 ng), a GnRH analog, [D-Ala^6]des-Gly^{10}-GnRH *N*-ethylamide (GnRHa, 1 μ*M*; Peninsula Laboratories), and a phosphodiesterase inhibitor (MIX, 400 μ*M*) were added immediately prior to culture. At selected times, portions of medium were removed and placed in a boiling water bath for 10 minutes, then stored frozen at −20°C for radioimmunoassay of cyclic AMP concentration (*4*). Extracellular concentrations of cyclic AMP were 90 percent or more of the total amount measured at the times indicated. Each point is the mean ± standard error of triplicate determinations from two to three separate experiments. *, $P < .05$; **, $P < .01$ (Student's *t*-test).

Upon stimulation with FSH, granulosa cells obtained from hypophysectomized rats with implanted diethylstilbestrol released cyclic AMP into the incubation medium (Fig. 1A). Concentrations of cyclic AMP were relatively constant from 3 to 24 hours of culture, and then increased from 24 to 48 hours. In contrast, cyclic AMP accumulated rapidly for 6 hours when the cells were cultured with FSH and the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (MIX) (Fig. 1B), then more gradually from 6 to 48 hours. The GnRH analog [D-Ala^6]des-Gly^{10}-GnRH *N*-ethylamide (GnRHa) completely inhibited the FSH-stimulated production of cyclic AMP from 24 to 48 hours when the granulosa cells were cultured without MIX. Instead, a decrease in cyclic AMP was detected from 24 to 48 hours of culture when GnRHa was present. At 3 and 6 hours of culture, GnRHa did not affect cyclic AMP levels in the presence or absence of MIX. However, the addition of MIX prevented the inhibitory action of GnRHa on cyclic AMP accumulation at 24 and 48 hours of culture. The action of MIX on GnRHa-induced inhibition of cyclic AMP formation by FSH-treated granulosa cells was concentration-dependent (Fig. 2). At 48 hours of culture, increasing concentrations of MIX above 1 μ*M* progressively abolished the inhibitory effect of the GnRH analog on FSH-stimulated cyclic AMP accumulation.

Since these data suggested that GnRHa affects cyclic AMP degradation, we examined the effect of GnRHa on phosphodiesterase activity in granulosa cells and ovarian tissue. To detect the effects of GnRHa on cyclic nucleotide catabolism in vitro, we added cyclic AMP to granulosa cells that had been cultured for 24 hours and measured the phosphodiesterase activity for the next 24 hours (Table 1). Both the high-affinity (1.0 μ*M* cyclic AMP substrate concentration) and the low-affinity (50 μ*M*) forms of phosphodiesterase activity were increased by GnRHa (Table 1). The GnRHa-activated phosphodiesterase is apparently a plasma membrane-associated enzyme, because the cyclic nucleotide does not enter cells readily and mimics peptide hormone action only when present at millimolar concentrations. However, the enzyme is not released during cell incubation, since granulosa cell medium contained negligible concentrations of phosphodiesterase activity (data not shown).

Studies in vivo showed that the phosphodiesterase activity of ovarian homogenates prepared from animals treated with FSH or FSH and GnRHa 24 hours previously was significantly greater when the FSH was administered with GnRHa rather than alone (Table 2). Once again, the activity of both the high- and low-affinity forms of the enzyme was increased by GnRHa.

The stimulation of phosphodiesterase activity by GnRHa was essentially a reversal of the inhibitory effect of FSH on cyclic nucleotide degradation. Treatment with FSH caused a decrease in basal phosphodiesterase activity, and concomitant treatment with GnRHa increased phosphodiesterase activity toward control levels. These results sug-

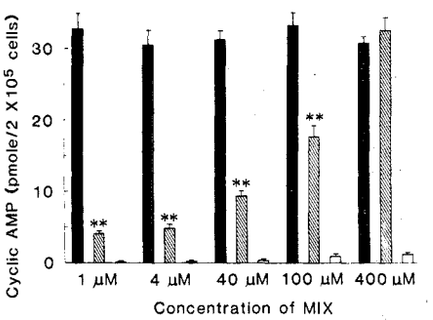

Fig. 2. Effect of MIX concentration on cyclic AMP accumulation during FSH and GnRHa treatment of granulosa cells cultured for 48 hours. Ovine FSH (250 ng, closed bars), FSH plus GnRHa (1 μ*M*, hatched bars), or no hormone (open bars), and varying concentrations of MIX were added to granulosa cells immediately prior to incubation. Each point is the mean ± standard error of triplicate determinations from two to three separate experiments. **, $P < .01$.

Table 1. Activation of cyclic AMP phosphodiesterase by GnRHa in rat granulosa cells in vitro. Intact granulosa cells were cultured for 24 hours with FSH or FSH and 0.1 μ*M* GnRHa. We then added [2,8-^{3}H]cyclic AMP; 2×10^5 count/min; 10 pmole; 32 Ci/mmole (New England Nuclear), plus unlabeled nucleotide to 1 μ*M* or 50 μ*M* concentrations. After incubation for 24 hours, portions of medium were removed and cyclic AMP metabolites were measured (*14*). Enzyme reactions were linear with respect to time and concentration. Values are the mean ± standard error for four to six experiments.

Treatment	Amount of cyclic AMP hydrolyzed (pmole/2 × 10^5 cells)	
	1 μ*M* cyclic AMP	50 μ*M* cyclic AMP
Control	426 ± 10	9304 ± 305
FSH	228 ± 11	4650 ± 510
FSH plus GnRHa	318 ± 39*	5770 ± 386*

*$P < .05$, Student's *t*-test (paired, FSH compared to FSH plus GnRHa).

gest that FSH not only increases cyclic AMP production by activating adenylate cyclase (*7*) but also maintains concentrations of cyclic AMP by inhibiting its catabolism. A similar effect of FSH on both adenylate cyclase and phosphodiesterase has been described in the Sertoli cell, the testicular counterpart of the granulosa cell (*8*). Thus, in both ovary and testis, regulation of target-cell function by FSH includes an inhibitory action on phosphodiesterase that serves to increase net cellular cyclic AMP production above that elicited only by activation of adenylate cyclase. Although the inhibitory effects of GnRH on ovarian development are well established (*1–6*), recent reports have indicated that some actions of GnRH involve stimulation of ovarian responses; for example, the inhibition of progesterone formation by GnRH is partly due to increased conversion of this steroid to a less active metabolite (*9*). Also, GnRH-induced prostaglandin synthesis occurs in rat granulosa cells (*10*), and GnRH increases meiotic maturation of rat oocytes (*11*). Activation of phosphodiesterase is a further example of a stimulatory effect of GnRH, in this case of a cellular process that inhibits gonadotropin-dependent ovarian function.

Since the ability of granulosa cells to produce cyclic AMP requires stimulation of cyclic nucleotide synthesis by FSH, an effect of GnRH agonists on adenylate cyclase activity could also account for the decreased cyclic nucleotide production in GnRHa-treated cells. Although GnRHa has little or no direct effect on adenylate cyclase activity in FSH-stimulated ovarian homogenates in short-term experiments (*5*), we have observed an inhibitory action on cyclic AMP synthesis during culture of GnRHa-treated granulosa cells. A progressive decrease in the ability of FSH to stimulate cyclic AMP synthesis occurred in GnRHa-treated cells at 24 and 48 hours of culture (Fig. 3), in conjunction with the increase in phosphodiesterase activity caused by the peptide (Table 1). Despite this decrease in adenylate cyclase activity by 48 hours of culture, accumulation of cyclic AMP by cells treated with FSH plus GnRHa in the presence of MIX (Fig. 1B) was maintained at or near the concentration in FSH-treated cells. Most of the FSH-stimulated increase in cyclic AMP accumulation had already occurred by 6 to 24 hours, and the increase of cyclic AMP brought about by MIX at 48 hours in GnRHa-treated cells largely reflects inhibition of further catabolism of the preformed nucleotide. These results demonstrate that both decreased production and increased degradation of cyclic AMP are responsible for the diminution of cyclic AMP concentrations in GnRHa-treated granulosa cell cultures.

Table 2. Activation of cyclic AMP phosphodiesterase by GnRHa in rat granulosa cells in vivo. Ovaries were obtained from hypophysectomized rats with implanted diethylstilbestrol. The rats had received FSH (7.5 μg) or FSH plus GnRHa (10 μg) 24 hours previously. The ovaries were homogenized and the 20,000*g* supernatant was used for measuring the reaction products as in Table 1 (*14*). Enzyme reactions were linear with respect to time and concentration. Values are the mean ± standard error for four to six experiments.

Treatment	Amount of cyclic AMP hydrolyzed (pmole/mg ovary)	
	1 μ*M* cyclic AMP	50 μ*M* cyclic AMP
Control	71 ± 7	1138 ± 105
FSH	57 ± 6	762 ± 36
FSH plus GnRHa	82 ± 10*	1140 ± 140†

*$P < .01$. †$P < .05$, Student's *t*-test (paired, FSH versus FSH plus GnRHa).

It is well known that cyclic AMP mediates the effects of gonadotropins on steroidogenesis in the ovary (*12*). Cyclic AMP also mediates the trophic actions of FSH in follicular granulosa cells, including formation of luteinizing hormone and prolactin receptors, cellular aggregation, microvillus formation, and differentiation of follicular granulosa cells (*1*). That GnRHa inhibits the stimulatory effects of exogenous cyclic AMP on granulosa cell function (*1*) is consistent with the present finding that GnRHa has a significant action on cyclic AMP degradation. These data emphasize the regulatory role of cyclic AMP in FSH action, and the necessity for maintenance of physiological levels of cyclic AMP in granulosa cell differentiation. Thus, it is possible that GnRH-mediated modulation of cyclic AMP metabolism could be a determinant of granulosa cell maturation and regression. Recent observations suggest that a GnRH-like factor exists in the ovary (*13*), raising the possibility that such peptides could influence gonadal function. Our data indicate that a regulatory action of GnRH-like peptides in the ovary is exerted at the level of adenylate cyclase and phosphodiesterase activation, and that inhibition of cyclic AMP responses to gonadotropic stimulation accounts for many of the direct antigonadal effects of GnRH agonists.

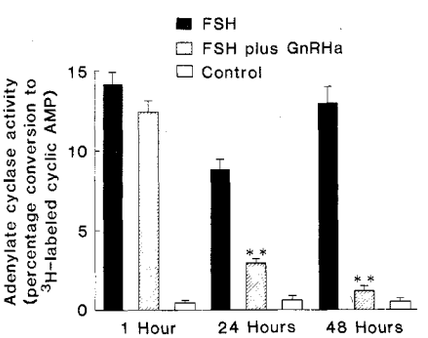

Fig. 3. Effect of GnRHa on FSH-stimulated adenylate cyclase activity during culture of 2×10^5 granulosa cells for 48 hours. Freshly prepared cells and cells cultured for 24 or 48 hours with FSH (250 ng), FSH plus GnRHa (0.1 μ*M*), or no hormones were washed twice with medium and incubated for 3 hours with 20 μCi of [^{3}H]adenine (15 Ci/mmole; New England Nuclear) to label intracellular pools of adenosine triphosphate. The cells were then washed twice and incubated with hormones in the presence of 1.0 m*M* MIX for 1 hour. Conversion of [^{3}H]ATP to ^{3}H-labeled cyclic AMP was measured as described (*15*). The uptake and conversion of [^{3}H]adenine to [^{3}H]ATP was similar after FSH and FSH plus GnRHa treatments (data not shown). Each point is the mean ± standard error of triplicate determinations from two experiments. **, $P < .01$.

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