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SHORT COMMUNICATION

Differing Specificities in the Desensitization of Ovarian Adenylate Cyclase by Epinephrine and Human Chorionic Gonadotropin

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SUMMARY

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Adenylate cyclase activity of luteinized ovaries from gonadotropin-primed immature rats was stimulated by luteinizing hormone (LH), epinephrine and prostaglandin E_1. Treatment of primed rats with human chorionic gonadotropin (hCG) caused a time-dependent loss of the adenylate cyclase response to LH, and the enzyme also became refractory to stimulation by epinephrine. Conversely, treatment of primed rats with epinephrine caused a time-dependent loss of the adenylate cyclase response to epinephrine, but the enzyme remained responsive to LH. The refractoriness of adenylate cyclase to epinephrine after desensitization by hCG or epinephrine was not reversed by Gpp(NH)p. These studies show that "cross-desensitization" of epinephrine-responsive adenylate cyclase activity in the ovary is induced by LH-receptor interaction, whereas β-adrenergic desensitization causes more specific refractoriness to epinephrine with no change in the LH receptor activation mechanism. This suggests the existence of fundamental differences in mechanisms of adenylate cyclase activation and/or desensitization by catecholamines and glycoprotein hormones.

INTRODUCTION

The biological actions of peptide hormones are known to depend, among other factors, upon the previous hormonal state of the target tissue. Frequently, treatment with the homologous hormone *in vivo* or *in vitro* causes a subsequent decrease or loss of the target-cell response to the hormone. Thus, administration of hCG[1] to rats is followed by diminished steroid responses in the testis (1–3) or ovary (4) during subsequent stimulation by gonadotropin *in vitro*. Such decreased steroid responses are associated with impaired hormonal activation of adenylate cyclase (5, 6), which in turn appears to arise from changes initiated by occupancy of a small proportion of the LH receptors (6). Similar changes have been observed in the function of the testis (7, 8) and ovary (9) due to gonadotropin receptor loss caused by elevation of endogenous LH on administration of LHRH and its analogues. Although the refractory state in gonadotropin-desensitized ovaries and testes is associated with a true loss of LH receptors after several hours (1, 6, 10), in the early stage of desensitization there is no change in the *total* receptor population, or even a transient increase in binding sites (6, 11). During the initial phase of hormone occupancy there is an early decrease in *free*

[1] The abbreviations used are: hCG, human chorionic gonadotropin; LH, luteinizing hormone; PMSG, pregnant mare serum gonadotropin; PGE_1, prostaglandin E_1.

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receptors, particularly at high doses of hCG, followed by the development of a state in which the hormone-receptor-adenylate cyclase complex is refractory to further stimulation by LH or hCG. During studies on the gonadotropin-induced desensitization of adenylate cyclase in the luteinized ovary, the loss of enzyme responses to LH was noted to be accompanied by a marked decrease in the response to epinephrine (12). In the present report, the phenomenon of cross-desensitization between gonadotropin and epinephrine regulation of ovarian adenylate cyclase was examined in the luteinized rat ovary, and shown to be assymetrical in that epinephrine-induced loss of the β-adrenergic responses did not alter the LH receptor-mediated activation of adenylate cyclase.

Ovarian luteinization was induced in immature female rats by sequential administration of PMSG and hCG as described previously (13). Eight days after PMSG/hCG treatment, the primed rats received either 20 μg of hCG or 1 mg of epinephrine by subcutaneous injection and were killed 1 to 3 hours later. Ovaries were rapidly removed and stored in liquid nitrogen until assayed for adenylate cyclase activity and LH receptor content by methods described in the figure legends.

In homogenates of control luteinized ovaries, epinephrine and LH produced about a five-fold maximum stimulation above the basal adenylate cyclase activity, as shown in Fig. 1. In animals treated with 20 μg of hCG before sacrifice, there was a time-dependent decrease in the ability of LH to stimulate the enzyme, which became maximally refractory in 3 hours as previously described (13). In the ovarian tissue of such hCG-treated animals, refractoriness of adenylate cyclase to LH was accompanied by loss of the stimulating effect of epinephrine.

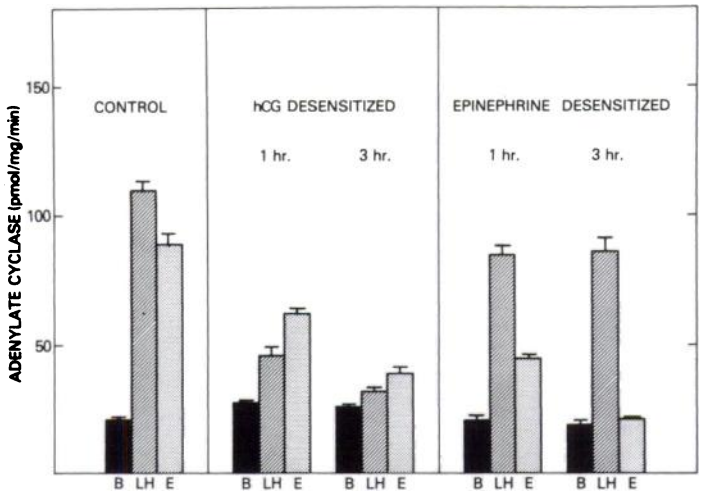

FIG. 1. *Effects of treatment with epinephrine or hCG on the hormonal sensitivity of ovarian adenylate cyclase*

Immature female rats were primed with 50 IU of PMSG followed after 65 hours by 25 IU of hCG. Eight days following the first injection, when the ovaries were fully luteinized, the rats were injected subcutaneously with a single dose of 20 μg of hCG or with 1 mg of epinephrine every hour. The animals were sacrificed 1 or 3 hours later and the ovaries were stored in liquid nitrogen until assayed for adenylate cyclase activity. Homogenates were prepared in a Pipes/hexylene glycol buffer pH 7.4, and assayed for adenylate cyclase activity for 10 min at 30°. The incubation mixture of 100 μl contained 2.5 mM ATP, [α^{32}P]ATP to give a specific activity of 17 cpm/pmole, creatine kinase/creatine phosphate as ATP-regenerating system, 5 mM $MgCl_2$, 1 mM cyclic AMP, 1 mM EDTA and 25 mM Tris-HCl pH 7.4. Epinephrine and LH were present at supramaximal doses of 10^{-5} M and 3.10^{-7} M, respectively. The incubation was terminated by 0.2 ml of 1 N $HClO_4$ and [^{32}P]cAMP was separated on Dowex/alumina columns (22). The adenylate cyclase reaction is linear under these conditions and the bars represent the mean ± SE. The data were calculated as picomoles of cyclic AMP formed per mg of protein per min and the results shown are from one of three similar experiments.

In other studies (12), the decreasing responses of adenylate cyclase to LH and epinephrine in hCG-treated animals were closely correlated with respect to time and desensitizing dose of hCG. In contrast, in rats treated with epinephrine, there was only a small decrease in the LH stimulation of ovarian adenylate cyclase activity at times when the epinephrine stimulation was partially decreased (1 hour) and completely lost (3 hours), as shown in Fig. 1, right panel. In the hCG- and epinephrine-treated animals, there was no increase in basal enzyme activity above that of the untreated animals.

In frog erythrocyte membranes, desensitization of β-adrenergic receptors is reversible by treatment with Gpp(NH)p (14), whereas in whole cells guanine nucleotides were only weakly effective in restoring receptor concentration (15). When the effect of the guanyl nucleotide was examined in the normal and epinephrine-desensitized ovary, as shown in Fig. 2, Gpp(NH)p stimulated both basal enzyme activity and the responses to LH and epinephrine in control ovaries. In the epinephrine-desensitized ovary, the stimulating effect of epinephrine was lost in the presence or absence of Gpp(NH)p, while the enzyme activation by LH was similar to that observed in the control ovary. Thus, Gpp(NH)p does not restore the responsiveness of adenylate cyclase to either LH (6) or epinephrine in ovaries desensitized by the corresponding hormone.

In contrast, the stimulating action of PGE_1 on ovarian adenylate cyclase was not affected by desensitization with either ligand, as shown in Fig. 3. Whereas pretreatment with hCG or epinephrine caused loss of both LH and epinephrine responses, or only the epinephrine response, respectively, there was no change in the action of PGE_1. It is possible that the PGE_1 receptor and adenylate cyclase are located in a different set of cells than those which are sensitive to LH and epinephrine. In this experiment, as described in the legend, the number of LH receptors was determined under both conditions of desensitization. The binding capacity of the control ovarian tissue was 224 fmole/mg of protein, and was reduced

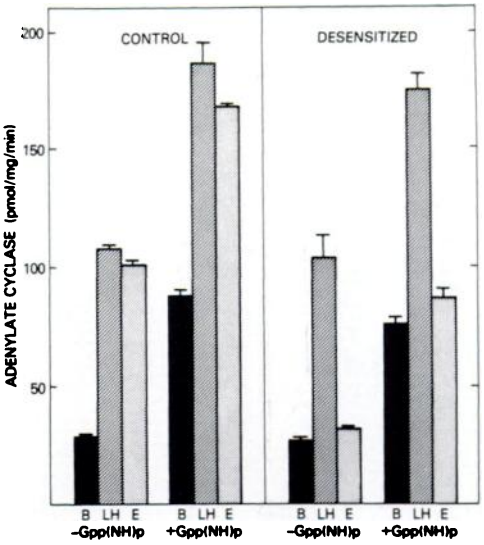

FIG. 2. *Effects of Gpp(NH)p on adenylate cyclase activity in the epinephrine-desensitized rat ovary*

Gonadotropin-primed rats were prepared as described in the legend to Fig. 1 and treated with 1 mg doses of epinephrine at zero time and after 2 hours. The animals were sacrificed at 3 hours and ovaries were homogenized and assayed for adenylate cyclase activity with additions at the following concentrations: LH, 3.10^{-7} M; epinephrine, 10^{-5} M; and Gpp(NH)p, 10^{-4} M. The bars represent the mean ± SE, and the data shown are representative of two similar experiments.

to 136 fmole/mg in the hCG-treated rats. In contrast, the LH receptor content remained at 238 fmole/mg in the epinephrine-treated animals. Thus, the full adenylate cyclase response to LH in the epinephrine-treated animal occurred with no change in the population of LH receptors, while loss of the LH response in the hCG-treated animals was accompanied by a 50% fall in the number of available LH receptors. Although the later stages of desensitization are accompanied by a net loss of receptors which correlates with the loss of LH stimulation of adenylate cyclase (at 1 to 3 days) in the ovary (16), the acute phase of desensitization occurs without a net loss of receptor sites (6, 11). Furthermore, as indicated here, adenylate cyclase was refractory to stimulation even when about one-half of the LH receptors were in the unoccupied state.

In other studies in which epinephrine was

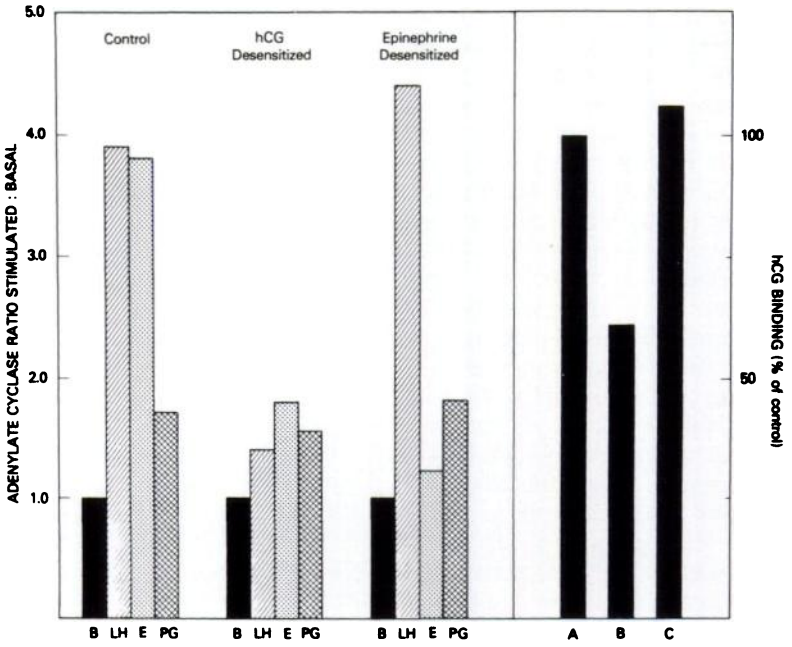

FIG. 3. *Comparison of LH receptor binding capacity and adenylate cyclase responses to LH, epinephrine, and PGE$_1$ in hCG- and epinephrine-desensitized rat ovary*

Gonadotropin-primed female rats were treated with a single dose of 20 μg of hCG or 1 mg of epinephrine at zero time and 2 hours, and sacrificed at 3 hours. Adenylate cyclase was assayed as described in the legend to Fig. 1, and LH binding capacity was determined by incubation of 3 aliquots in duplicate with ^{125}I-hCG of specific activity 1430 cpm/fmole for 16 hours at 24°. Nonspecific binding was determined for each aliquot by incubating in duplicate in the presence of 50 IU hCG. The binding capacity, calculated as the amount of hCG bound per mg of protein, was 234 ± 3.8 (SE) and 238 ± 4.0 (SE) fmole in the control and epinephrine-desensitized ovaries, and was reduced to 136 ± 1.3 (SE) fmole in the hCG-desensitized tissue. In the hCG binding data, A, B and C refer to control, hCG-desensitized and epinephrine-desensitized ovaries, respectively.

injected intravenously (2 μg/min) under pentobarbitone anaesthesia, no desensitization of the epinephrine stimulation of adenylate cyclase was seen after a 30 minute infusion. This suggests that the loss of responsiveness observed after subcutaneous treatment with epinephrine, which was partial at 1 hour and complete at 3 hours (Fig. 1), involves slow changes in the cell membrane, as has also been suggested for the loss of LH action following desensitization with hCG (6).

These experiments in the luteinized ovary demonstrate that cross-desensitization of adenylate cyclase by different hormonal ligands can be unidirectional, with differing specificities during the phase of desensitization. Whereas rats treated with hCG exhibit loss of adenylate cyclase responsiveness to both LH and epinephrine, rats treated with epinephrine lose only the enzyme response to epinephrine, and enzyme stimulation by LH is not reduced. These results suggest that the receptors for LH and epinephrine can interact with the same pool of adenylate cyclase. This conclusion is supported by the finding that the combined effects of epinephrine and LH upon adenylate cyclase (12, 17) and progesterone (12) production in isolated luteal cells did not exceed the effect of either hormone alone. If the two receptors were in different cell populations, or acting via independent adenylate cyclase pools, these responses would be expected to be additive. There appears to be no direct interaction between the β-receptor and the LH receptor, since the effects of epinephrine were blocked by propranolol but not phentolamine, while neither antagonist had any

effect on the actions of LH (12).

During the later stages of hCG-induced desensitization between one and four days (16), there is a close correlation between LH-responsiveness and receptor number in the luteinized ovary. However, during the acute phase 3 hours after hCG treatment, there was complete loss of the adenylate cyclase response to both LH and epinephrine when about 50% of the total LH receptors were still unoccupied. No significant loss of β-receptor sites has been detected in the hCG-desensitized ovary during refractoriness to epinephrine stimulation (12). Furthermore, at this early stage, fluoride stimulation of adenylate cyclase was also reduced, suggesting that the hormone-receptor-adenylate cyclase complex was completely refractory (16). During this phase, hormonal occupancy of the residual LH receptors, or the full complement of β-receptors, or addition of fluoride, could not stimulate the enzyme.

In contrast to the actions of LH, the loss of β-adrenergic stimulation of adenylate cyclase in the epinephrine-desensitized ovary was not accompanied by decreased responsiveness of the enzyme to LH. As might be anticipated from this observation, no change in LH receptor content of the ovary was found after desensitization with epinephrine. Therefore, hCG-induced desensitization appears to lead to more extensive changes in the cell membrane, perhaps with nonspecific effects on other ligand actions, whereas epinephrine produces changes that are much more specific. In this regard, hCG-induced loss of ovarian receptors has been found to be associated with a process of internalization or endocytosis (18, 19). The membrane turnover associated with these processes might render adenylate cyclase insensitive to adrenergic stimulation without necessarily causing a decrease in the number of β-receptors. In contrast, desensitization of the β-receptor probably occurs via a process that does not involve membrane turnover, and therefore does not interfere with LH receptor activation of adenylate cyclase. The considerable difference in the rates of hormone-receptor interaction and dissociation between the adrenergic (14, 20) and gonadotropin (21) receptors also suggests marked differences in the mechanism of action of the two ligands, and may contribute to the prolonged refractoriness observed in the hCG-desensitized target tissue.

Non-specific or cross-desensitization has been recently described in astrocytoma cells (20), in which incubation with either isoproterenol or PGE_1 caused marked loss of the cyclic AMP response to the homologous hormone and partial desensitization to the heterologous hormone. In the astrocytoma cell, the loss of isoproterenol response induced by isoproterenol or PGE_1 was accompanied by little or no loss of β-receptors, respectively. Although cross-desensitization was clearly seen at the level of cyclic AMP production in intact cells, the effect was not observed in direct studies of adenylate cyclase activity in broken cells. In the ovarian system, the LH-induced cross-desensitization to epinephrine in the isolated luteal cells (12) is also evident in adenylate cyclase assays performed in broken cell preparations during the present study. These findings support the concept that hormone-induced refractory states of adenylate cyclase are rapidly formed after hormone-receptor interaction, and do not necessarily involve a significant loss of total receptor number. The extent to which adenylate cyclase forms the refractory state, and the rate of reversal of the desensitized condition, will determine the subsequent hormonal responsiveness of the tissue.

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