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[REVIEW]

GnRH as a Cell Proliferation Regulator: Mechanism of Action and Evolutionary Implications

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ABSTRACT—Gonadotropin-releasing hormone (GnRH) is well known as the central regulator of the reproductive system through its stimulation of gonadotropin release from the pituitary. Studies on GnRH have demonstrated that GnRH has both stimulatory and inhibitory effects on cell proliferation depending on the cell type; however, the mechanisms of these effects remain to be elucidated. Against this background we used four human cell lines, TSU-Pr1, Jurkat, HHUA and DU145, and newly found that GnRH increased or decreased the colony-formation depending on the cell line. Moreover, we demonstrated that the stimulatory and inhibitory effects of GnRH exhibit distinct ligand selectivities. In order to investigate the molecular basis of these phenomena, analyses of the expression of human GnRH receptors were performed and, moreover, the effects of GnRH were analyzed under conditions in which human GnRH receptors were knocked down by the technique of RNA interference. Consequently, it was found that human type II GnRH receptor, which had been suspected of being nonfunctional because of alterations in its sequence, is involved in the effects of GnRH on cell proliferation. In this article, the influence of the autocrine activities of the cells is also reviewed, focusing on the characteristics of substances secreted from the four cell lines. Based on recent studies of GnRH and its receptors and our up-to-date findings, the evolutionary implications of GnRH action are discussed.

Key words: GnRH, GnRH receptor, cell proliferation, colony-formation, reproduction

INTRODUCTION

GnRH was originally identified as a hypothalamic decapeptide which promotes gonadotropin secretion from pituitary gonadotropes and was named gonadotropin-releasing hormone (Matsuo *et al*., 1971; Amoss *et al*., 1971). Thereafter, multiple GnRH isoforms and multiple types of GnRH receptors were reported in a wide range of organisms in addition to vertebrates (Millar *et al*., 2004) and broad distribution of them in extrapituitary tissues and organs has been demonstrated (Klausen *et al*., 2002; Millar, 2003; Ikemoto *et al*., 2003, 2004). Through these observations the study of extrapituitary GnRH systems has become one of the important topics regarding the physiological significance of GnRH. The concept of extrapituitary GnRH systems is also important in terms of the evolution of the reproductive system. Now that the existence of GnRH has been confirmed in invertebrates, which have no pituitary gland, it is suggested that in these organisms GnRH acts directly on the gonads (Terakado, 2001; Kusakabe *et al*., 2003). In our previous studies (Kogo *et al*., 1995, 1999a, b; Park *et al*., 1999), GnRH receptor mRNA expression was demonstrated in the rat ovary, which strongly suggests the idea that GnRH is involved in the induction of follicular atresia and ovulation (Table 1). There have also been other studies suggesting that GnRH directly controls gonadal function. (Gobetti *et al*., 1992; Fasano *et al*., 1995; Takekida *et al*., 2003; Sifer *et al*., 2003), however clear evidence supporting this suggestion has not been obtained yet. Moreover, in addition to the direct effects on gonads, it has been reported that GnRH may act as a neuromodulator or an immunomodulator. (Eisthen *et al*., 2000; Oka, 2002; Ford *et al*., 2003; Azad *et al*., 1997; Enomoto *et al*., 2001; Chen *et al*., 2002). Although a number of studies have been performed on these effects of GnRH, little is known about the molecular mechanisms and physiological settings in which GnRH exerts its activities in

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Table 1. GnRH receptor mRNA expression on the rat ovary.

Tissues		Strength and localization of the signal	Remarks
Primordial follicles		No siganal	
Granulosa cells of healthy follicles	Preantral follicles	Weak	Throughout the estrous cycle
	Early antral follicles		
	Medium antral follicles	Peripheral part of granulosa layer*	
		Became prominent	Mainly diestrous day 1
	Large antral and Graafian follicles	Very strong	Diestrous day 2 and proestrus
	Ovulated follicles (on estrus 0:00)	Strong	
Theca cells of healthy follicles		None, sometimes very weak	
Corpora lutea	First generation	Moderate	
	Second generation	Degenerative change	
	Third generation		
Atretic follicles	Granulosa cells	Very strong throughout their atretic process	
	Theca cells	Moderate in progressed atretic follicles	
Interstitial cells		Moderate	

* Expression of the mRNA become prominent in peripheral part of granulosa cells, where the nuclei of the positive cells were elongated. No significant signals were detected in the inner part of the granulose layer.

extrapituitary tissues or organs.

There are interesting *in vitro* studies about the diverse functions of GnRH. GnRH promotes or inhibits cell proliferation depending on the cell type. As for the promotive effects of GnRH, effects on thymocytes, splenocytes, and lymphocytes have been reported (Marchetti *et al*., 1989; Batticane *et al*., 1991; Azad *et al*., 1997). Regarding inhibitory effects, effects against hormone-related tumors are well known, and these effects are now applied clinically (Schally, 1999; Grundker *et al*., 2002). These opposite effects of GnRH on cell proliferation are very interesting, but it has remained unknown whether GnRH has such activities in physiological settings, mainly because most previous studies were performed using cancer cell lines and pharmacological doses of GnRH. However, recently, the effects of GnRH on the cell proliferation of non-tumor cells, as well as on cell adhesion, cell migration, and cytoskeletal remodeling have been reported (Chen *et al*., 2002; Romanelli *et al*., 2004; Davidson *et al*., 2004) and the physiological roles of the effects of GnRH on cell proliferation are being reconsidered.

In fact, there is evidence for the effects of GnRH on gonadotrope cell proliferation (Sakai *et al*., 1988; Kakar *et al*., 1997; Miles *et al*., 2004), and thus GnRH may originally act as one of the cell growth regulators of gonadotropes. Moreover, there is a possibility that the activities of GnRH that alter their direction of effects depending on the cell type underlie the diverse physiological functions of GnRH. We have focused on the two opposite effects of GnRH on cell proliferation, particularly the relationship between these effects and the diverse functions of GnRH, and investigated the molecular mechanisms of the effects of GnRH on cell proliferation. So far, we have found stimulatory and inhibitory activities of the physiological dose of GnRH on the colony-formation of cells using an originally established method, the colony-forming efficiency assay (Enomoto *et al*., 2001 and 2004a, b). The experimental models used in our studies are TSU-Pr1 (from human prostatic carcinoma, Iizumi *et al*., 1987), Jurkat (from human mature leukemia, Gills and Watson, 1980), DU145 (from human prostatic carcinoma, Mickey *et al*., 1977; Stone *et al*., 1978), and HHUA (from human endometrial carcinoma, Ishiwata *et al*., 1984) cell lines. Recently, interesting findings about the mechanisms of the two opposite effects of GnRH have been obtained by adaptation of the technique of RNA interference to our original assay system (Enomoto *et al*., 2004b). Moreover, we have obtained strong evidence for the influence of autocrine activities of the cells on the GnRH effects (Enomoto *et al*., 2001, 2004a). In this article, our up-to-date findings about the mechanisms of the stimulatory and inhibitory effects of GnRH will be reviewed, focusing on human GnRH receptors and the influence of autocrine activities of the cells on the GnRH effects. The evolutionary implication of the GnRH activities on cell proliferation will be also discussed.

1. Effects of GnRH on colony-formation

1-1. Stimulatory and inhibitory effects of GnRH

A number of studies have demonstrated that various carcinomas of breast, ovary, endometrium, prostate, pancreas and liver origin respond to GnRH and its analogues (Schally, 1999). It has also been reported that treatment of

thymocytes, splenocytes, and lymphocytes with GnRH and its analogues increased their proliferative activity (Marchetti *et al*., 1989; Batticane *et al*., 1991; Azad *et al*., 1997). In these studies the effects of GnRH on cell proliferation were investigated by measuring tumor weight and/or volume, the rate of cell proliferation, or ^{3}H-thymidine incorporation (Redding and Schally, 1983; Sharoni *et al*., 1989; Pati and Habibi, 1995; Dondi *et al*., 1998). However, to our knowledge, there have been no studies that compared stimulatory with inhibitory effects. One of the reasons for this was the lack of a method that makes it possible to detect both effects sensitively. Thus, we first established an original assay, the colony-forming efficiency assay (Enomoto *et al*., 2001). The colony-forming efficiency assay is based on limiting dilution analysis with minor modifications. The detailed procedure was described in our previous report (Enomoto *et al*., 2001).

Fig. 1 shows the effects of GnRH-I on the colony-forming efficiency. The slope of the linearly regressed line represents the colony-forming efficiency. In TSU-Pr1 and Jurkat cells, GnRH-I increased the colony-forming efficiency, whereas, in DU145 and HHUA cells, the colony-forming efficiency was decreased by GnRH-I. We also examined the effects of GnRH-I on the rate of cell proliferation and confirmed that the response to GnRH of each cell line was the same as that on colony-forming efficiency. This fact suggests that the colony-forming efficiency assay is useful for studying the effects of GnRH on cell proliferation. Moreover, it is very interesting that TSU-Pr1 and DU145, which are derived from human prostatic carcinoma, exhibited the opposite responses to GnRH stimulation.

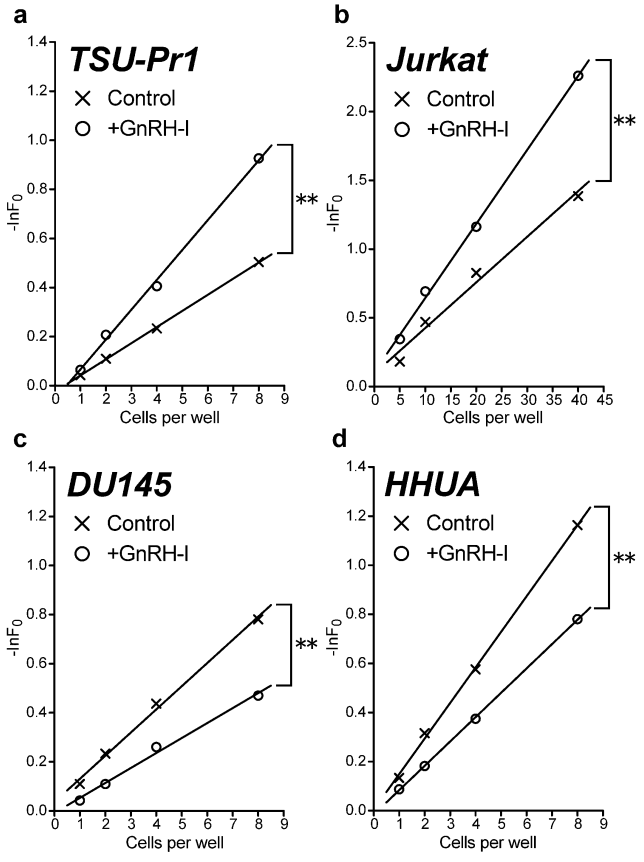
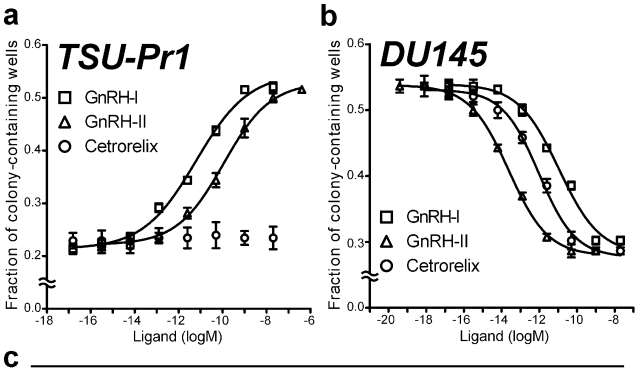


Fig. 1. Effects of GnRH-I (final concentration, 100 pM) on the colony-forming efficiency of TSU-Pr1 (**a**), Jurkat (**b**), DU145 (**c**), and HHUA (**d**) cells (**p<0.01). The number of cells plated per well was 1, 2, 4, and 8 cells in the cases of TSU-Pr1, DU145, and HHUA cells and 5, 10, 20, and 40 cells in the case of Jurkat cells.

1-2. Distinct ligand selectivities of the stimulatory and inhibitory effects

As described above, we succeeded in detecting the stimulatory and inhibitory effects of GnRH on the colony-forming efficiency. To clarify the characteristics of these effects, ligand selectivities were investigated using the four cell lines. As GnRH ligands, we used GnRH-I and II and Cetrorelix (synthetic type I GnRH receptor antagonist) (Reissmann *et al*., 2000). Humans GnRHs consist of GnRH-I and II (Matsuo *et al*., 1971; Amoss *et al*., 1971; Miyamoto *et al*., 1984). The results revealed that the stimulatory and inhibitory effects exhibited distinct patterns (Fig. 2). Fig. 2a and 2b show representative results. In Jurkat cells, the pattern is similar to that of TSU-Pr1 cells, and in HHUA the pattern is similar to that of DU145 (data not shown). In positively responding cell lines (TSU- Pr1 and Jurkat) GnRH-I was more effective (about 10 fold) than GnRH-II, and Cetrorelix had no effect. On the other hand, in negatively responding cell lines (DU145 and HHUA) GnRH-II was much more effective (1,000 to 10,000 fold) than GnRH-I. Interestingly, Cetrorelix behaved as an agonist. Fig. 2c lists the EC_{50} values of all nonlinearly fitted curves. Based on the classical ligand-receptor theory, this result strongly suggests



c

	EC50 [logM]		
	GnRH-I	GnRH-II	Cetrorelix
TSU-Pr1	-11.21±0.145	-9.95±0.191	no effect
Jurkat	-12.61±0.244	-11.42±0.369	no effect
DU145	-10.96±0.170	-13.62±0.115	-12.02±0.164
HHUA	-11.77±0.248	-15.57±0.249	-14.05±0.183

Fig. 2. Ligand selectivities for colony-formation of TSU-Pr1 (**a**) and DU145 (**b**) cells. The cell densities were 8 cells per well. The abscissa and ordinate represent the concentration of GnRH ligand and the fraction of colony-containing wells (the number of colony-containing wells per total wells), respectively. Results are expressed as means±SE (n=4). **c.** EC50 values of all nonlinearly fitted curves in TSU-Pr1, Jurkat, DU145, and HHUA cells.

that the stimulatory and inhibitory effects of GnRH are mediated by different GnRH receptors. Thus, we investigated this possibility as described in the following chapter.

2. Two types of human GnRH receptors

2-1. Expression of human GnRH receptors

Among the human GnRH receptors, type I (hGnRHR-1) and II (hGnRHR-2) GnRH receptors are known. Type I receptor was cloned first and has been well characterized (Kakar *et al*., 1992), and recently the genomic loci encoding the human type II GnRH receptor has been cloned (Faurholm *et al*., 2001). However, previous studies revealed a puzzling fact about the human type II GnRH receptor gene. The protein coding sequences contain a frameshift mutation and a TGA stop codon, compared with other primate type II GnRH receptor sequences that were demonstrated to be functional (Faurholm *et al*., 2001; Neill, 2002; Morgan *et al*., 2003). So far, no full-length sequence of a human type II receptor that is translated into protein has been discovered.

In 2001, Millar *et al*. demonstrated that human type I GnRH receptor and marmoset type II GnRH receptor, which has 90% amino acid identity with the deduced human type II GnRH receptor amino acid sequence, exhibited distinctly different patterns of ligand selectivity, as shown Fig. 2. This result strongly suggests that human type II GnRH receptor is functional as a receptor, and we first presumed that the distinctly different patterns of ligand selectivity between the stimulatory and inhibitory effects should be due to the expression of a different GnRH receptor subtype. Thus we performed expression analyses of human GnRH receptors in the four cell lines. For hGnRHR-1, a single PCR product was amplified with one primer set in all four cell lines (upper panel in Fig. 3). In contrast, for hGnRHR-2, two PCR products were amplified with one primer set in all four cell lines (lower panel in Fig. 3). The results of direct sequencing demonstrated that the longer PCR product was identical with the human type II GnRH receptor (GeneBank accession No. AY077708) (Van Biljon *et al*., 2002), and the lower one was identical with the human type II GnRH receptor splice variant (hGnRHR-2v) (GeneBank accession No. AY081843) (Neill, 2002). As shown in Fig. 3, all four cell lines expressed hGnRHR-1, 2, and 2v, which suggests that the opposite responses to GnRH stimulation may not be due to the differences of expressed GnRH receptor subtypes.

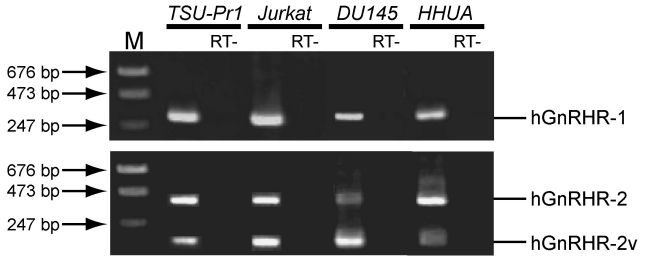


Fig. 3. Expression of human GnRH receptor 1 (the upper panel), 2 and 2 variant (the lower panel) in TSU-Pr1, Jurkat, DU145, and HHUA cells. RT- are negative controls in which non-reverse transcribed mRNA of each cell line was used as the template. The left-hand lane contains the molecular marker.

2-2. Involvement of each human GnRH receptor in the effects of GnRH

In the expression analyses, no clear differences between positively responding cell lines (TSU-Pr1 and Jurkat) and negatively responding cell line (DU145 and HHUA) were observed. Next we examined the effects of GnRH-I and II and Cetrorelix on colony-forming efficiency under the condition that the expression of each of the human GnRH receptors was suppressed individually by the technique of RNA interference (RNAi) using p*Silencer* 2.0-U6, a short hairpin interfering RNA (shRNA) expression vector (Ambion Inc., Austin, TX). This technique efficiently induces sequence-specific mRNA degradation and thereby suppresses the target gene expression (Brummelkamp *et al*., 2002; Hannon, 2002; Shi, 2003; Dykxhoor *et al*., 2003), and thus the roles of human type II GnRH receptor and its splice variant can be investigated even if they don't function as proteins. Table 2 shows the summary of the complete results. As a negative control, we used the p*Silencer* negative control vector encoding a shRNA whose sequence is not found in the mouse, rat, or human genome databases (Ambion Inc., Austin, TX), and the resultant colony-forming efficiencies were the same as those of non-transfected cells (data not shown).

As shown in Table 2, when hGnRHR-1 was knocked-down, all three GnRH ligands failed to show significant effects and the colony-forming efficiencies were similar to those of the non-transfected cells. When hGnRHR-2 was knocked-down, GnRH-II and Cetrorelix had no significant effects, and only GnRH-I was significantly effective. For hGnRHR-2v, quite interesting and remarkable results were obtained.When hGnRHR-2v was knocked-down, the direction of response to the stimulation of GnRH-I and II in TSU-

Table 2. Summary of the results when each human GnRH receptor was knocked down.

		TSU-Pr1	*Jurkat*	*DU145*	*HHUA*
non-transfected cells	GnRH-I	+	+	–	–
	GnRH-II	+	+	–	–
	Cetrorelix	n.e.	n.e.	–	–
hGnRHR-1 knocked down	GnRH-I	n.e.	n.e.	n.e.	n.e.
	GnRH-II	n.e.	n.e.	n.e.	n.e.
	Cetrorelix	n.e.	n.e.	n.e.	n.e.
hGnRHR-2 knocked down	GnRH-I	+	+	–	–
	GnRH-II	n.e.	n.e.	n.e.	n.e.
	Cetrorelix	n.e.	n.e.	n.e.	n.e.
hGnRHR-2v knocked down	GnRH-I	–	–	–	–
	GnRH-II	–	–	–	–
	Cetrorelix	–	–	–	–

Normal cells means non-transfected cells. +, significant stimulatory effect of GnRH ($p<0.05$); –, significant inhibitory effect of GnRH ($p<0.05$); n.e., no significant effect of GnRH.

Pr1 and Jurkat cells was reversed from a positive effect to a negative one, and Cetrorelix behaved as an agonist. To the best of our knowledge, this result is the first demonstration that a receptor can regulate the direction of the response to the ligand stimulation. As for DU145 and HHUA cells, no such alteration was observed upon the suppression of hGnRHR-2v. Although hGnRHR-2v mRNA is expressed in DU145 and HHUA cells, it appears to have no function in these cells. This fact may be due to the balance of the expression levels of hGnRHR-2 and 2v. In fact, as shown in Fig. 3, in DU145 and HHUA cells these levels are quite different, whereas in Jurkat and TSU-Pr1 cells they are much less different; however, further studies will be necessary to clarify this point.

Summarizing our results focusing on each receptor, hGnRHR-1 was indispensable for the effectiveness of all three GnRH ligands, and hGnRHR-2 was necessary for the effectiveness of GnRH-II and Cetrorelix. Furthermore, hGnRHR-2v plays a role in mediating the stimulatory effects of GnRH-I and II and the effectiveness of Cetrorelix. One possible explanation for these complicated results is that hGnRHR-2 and 2v directly interact with hGnRHR-1 and form hetero-dimers or oligomers. In fact, many recent studies have provided evidence that G-protein coupled receptors (GPCRs) can exist as either dimers or higher order oligomers (Milligan *et al*., 2004; Pfleger *et al*., 2004). Promotion or inhibition of signal transduction by ligand stimulation and alteration of G-protein selectivity by receptor dimerization have been also reported (AbdAlla *et al*., 2001a, b). Moreover, there is evidence that co-expression of GPCR pairs can generate distinct ligand-binding sites (Maggio *et al*., 1998). Fig. 4 shows one of the proposed structural models for GPCR receptor dimers, 5,6-domain swapped dimers (Gouldson *et al*., 1998). Each transmembrane alpha helix is represented as a circle. The hinge loop represents intracellular loop 3. As shown in this figure, another binding pocket is generated by receptor dimerization. This could account for our distinct patterns of ligand selectivities. We should also consider the possibility of cross-talk among intracellular signalings and functions as non-coding RNAs (Storz, 2002) of hGnRHR-2 and 2v mRNAs to modulate the signaling mediated by hGnRHR-1. However, in any case, further investigations of the human type II GnRH receptor and the splice variant will be required to elucidate the detailed mechanisms by which human type II GnRH receptor and its splice variant are involved in the effects of GnRH on cell proliferation.

3. Influence of autocrine activities of the cells on the GnRH effects

The colony-forming efficiency assay is a method that can examine cell proliferation at the low cell density (10 to

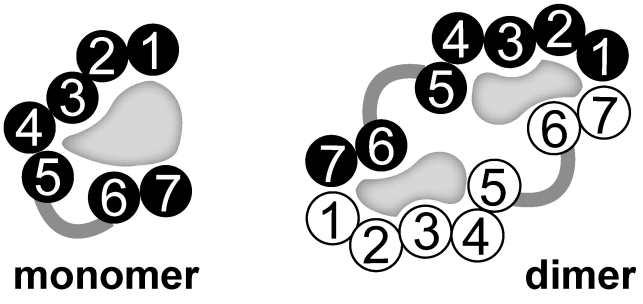


Fig. 4. Schematic models of the monomer and the 5,6-domain swapped dimer of G-protein coupled receptor. Each transmembrane alpha helix is represented as a circle. The hinge loop represents intracellular loop 3. The presumed binding pockets are shown as grey areas.

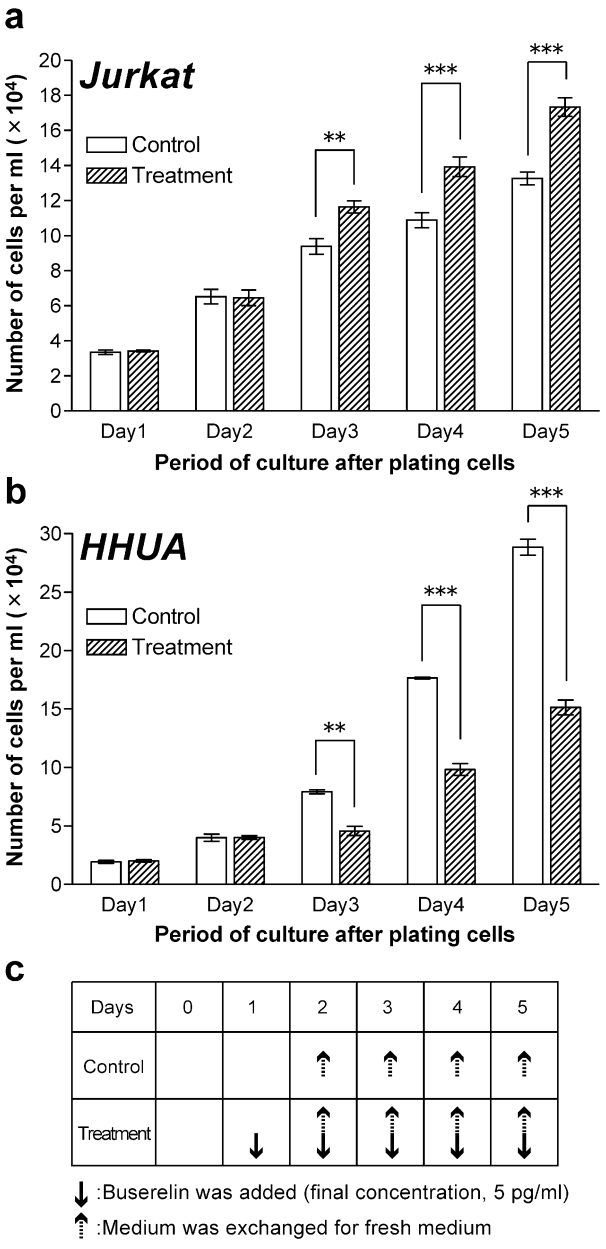


Fig. 5. Effects of Buserelin (final concentration, 5 pg/ml) on the rate of cell proliferation of Jurkat (**a**) and HHUA (**b**) cells when culture medium was exchanged every 24 hours. Results are expressed as means±SE (n=4; **p<0.01; ***p<0.001). The experimental schedule is illustrated in **c**.

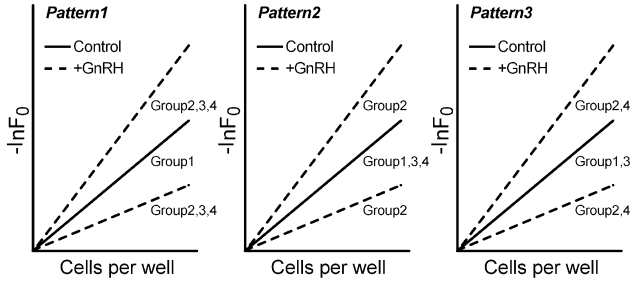
400 cells per ml). In our previous study, the effects of GnRH on the rate of cell proliferation at high cell density (20,000 cells per ml) were also investigated and interesting facts about the effects of GnRH were found. The assay was done as follows. At first, Jurkat and HHUA cells were inoculated in flat-bottomed 24-well plates (20,000 cells per ml). The day when cells were inoculated was defined as Day 0. Buserelin, a synthetic GnRH agonist was added (final concentration, 5 pg/ml) only after 24 hours or every 24 hours. In results, Buserelin had no significant effect on the rate of cell proliferation of either Jurkat or HHUA cells (data not shown). However, as shown in Fig. 5c, when Buserelin was added with daily exchange of the culture medium, the rate of cell proliferation was significantly increased or decreased in Jurkat or HHUA cells, respectively (Fig. 5a and 5b). From these results we presumed that the accumulation of substances secreted from the cells into the medium influenced the effects of GnRH on cell proliferation. To confirm this possibility, we measured the colony-forming efficiency in the presence of conditioned media of Jurkat, HHUA and TSU-Pr1 cells. Conditioned medium is the medium in which cells have been cultured for several days, and thus it contains substances secreted from cells during the culture period. The experimental groups prepared are shown in Fig. 6a. The detailed procedure of this experiment was described in our previous report (Enomoto *et al*., 2001).

As shown in Fig. 6b, the colony-forming efficiencies in the presence of conditioned media of Jurkat, HHUA and TSU-Pr1 cells were classified into three patterns. In the case of Pattern 1, in the presence of the conditioned medium, the colony-forming efficiency (Group 3 and 4) was nearly equal to that of Group 2, being independent of the addition of GnRH. In contrast, in the case of Pattern 2, the colony-forming efficiency in the presence of conditioned medium (Group 3 and 4) was nearly equal to that of Group 1, being independent of the addition of GnRH. In Pattern 3, the presence of the conditioned medium had no effect. Fig. 6c shows the comprehensive results based on the classification of the three patterns. These results indicate that the conditioned medium of Jurkat cells mimicked the GnRH activity, whereas that of HHUA cells inhibited it. In other words, Jurkat cells secrete a GnRH-like substance and HHUA cells secrete a GnRH-inhibiting substance in terms of the effects on cell proliferation. However, the conditioned medium of TSU-Pr1 cells inhibited the GnRH activity only in TSU-Pr1 cells themselves. In HHUA cells, it had no effect, whereas in Jurkat cells, it severely decreased the colony-forming effi-

a

Group	Fresh medium	Conditioned medium	GnRH
Group1	100%	—	—
Group2	100%	—	↓
Group3	50%	50%	—
Group4	50%	50%	↓

b



c

	Conditioned medium		
	Jurkat	HHUA	TSU-Pr1
TSU-Pr1	Pattern1	Pattern2	Pattern2
Jurkat	Pattern1	Pattern2	*
HHUA	Pattern1	Pattern2	Pattern3

*, The colony-forming efficiency was severely decreased and statistically analyzable data could not be obtained.

Fig. 6. Summary of the effects of conditioned media derived from Jurkat, HHUA and TSU-Pr1 cells on the colony-forming efficiencies. **a.** Experimental groups. **b.** Three patterns of the influence of conditioned media. **c.** Summarized results of the effects of each conditioned medium on the colony-forming efficiencies of Jurkat, HHUA, and TSU-Pr1 cells.

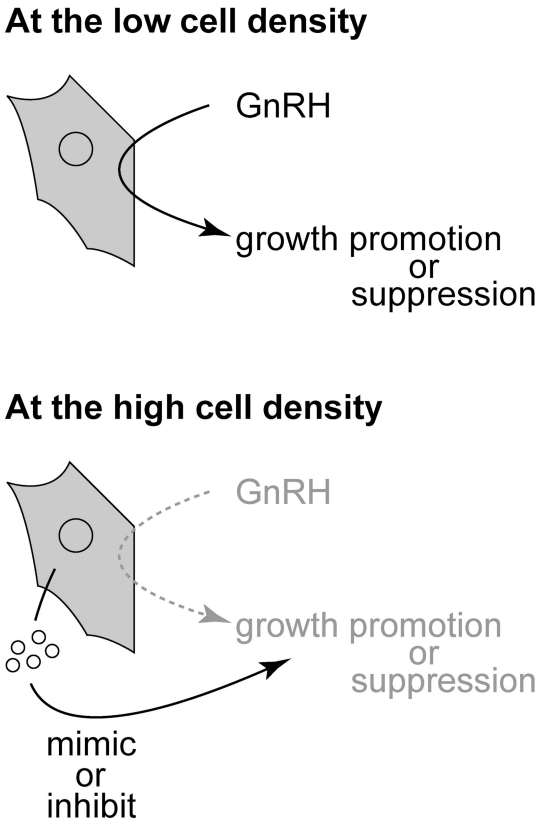


Fig. 7. Cartoon illustrating the response of cells simulated by GnRH at low and high cell density.

ciency and statistically analyzable data could not be obtained. Summarizing these results, the conditioned media of Jurkat and HHUA cells consistently affected the GnRH activities regardless of the cell type, whereas that of TSU-Pr1 had cell type-dependent effects. Our results suggest that each of these three cell lines secrete a distinct substance that affects GnRH activities, and these substances will be identified in future studies.

Our interpretation of the results introduced in this chapter is schematized in Fig. 7. At low cell density, GnRH promotes or suppresses cell proliferation depending on the cell type. In contrast, at high cell density autocrine factors of the cells mimic or inhibit the GnRH activity and the effects of GnRH are masked. From this point of view, the colony-forming efficiency assay is a sensitive method that can limit the effects of autocrine activity of the cells.

EVOLUTIONARY IMPLICATIONS

In this article, we reviewed our recent findings about the mechanisms of the stimulatory and inhibitory effects of GnRH on cell proliferation, focusing on the involvement of GnRH receptors. The detailed role of each receptor remains to be elucidated. In addition, we described the autocrine activities of the cells that affect the GnRH effects. Identification of these factors is important to clarify the effects of GnRH on cell proliferation *in vivo*.

Viewing the historical progress on studies of GnRH, we are reminded of one of the bioactive substances in the hypothalamus-pituitary-gonadal axis, namely activin. Activin was originally isolated based on its activity in stimulating follicle-stimulating hormone (FSH) release from the anterior pituitary (Ling *et al*., 1986; Vale *et al*., 1986). In addition to its endocrine function, activin has been found to possess various activities in different biological systems, e.g., erythroid differentiation, nerve cell survival, *Xenopus laevis* embryonic mesoderm induction, bone growth promotion, and somatostatin induction (Mathews, 1994). Subsequently, it was found that activin regulates a wide variety of cellular events, including cell proliferation, differentiation, and apoptosis. For example, in addition to endocrine function in the pituitary, activin also controls the activity of the hypothalamus and ovary, indicating that activin has profound autocrine and paracrine effects on the female reproductive system (Peng and Mukai, 2000). GnRH was originally identified as a hypothalamic decapeptide that promotes gonadotropin release, and thereafter a number of studies have strongly suggested that it has various physiological activities, such as neuromodulation, immunomodulation, and regulation of follicular atresia and ovulation. Moreover, it was demonstrated that GnRH regulates cell proliferation, cell migration, and cell attachment. Based on these similarities between activin and GnRH, the idea that GnRH may have roles in embryogenesis and morphogenesis occurs to us. In fact, there have been several reports suggesting that GnRH may play a substantial autocrine or paracrine role in human fertilization, early embryonic development, and implantation (Seshagiri *et al*., 1994; Casan *et al*., 1999; Raga *et al*., 1999). From these points of view, our studies of the molecular mechanisms of the GnRH actions on cell proliferation will provide important clues for clarifying the physiological roles of GnRH, particularly in embryogenesis and morphogenesis. Moreover, extending our findings about human GnRH receptor signalings to other species will lead to a comprehensive understanding of the GnRH system, including the GnRH system in invertebrates, and clarification of the evolution of the reproductive system.

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