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Immortalized Luteinizing Hormone-Releasing Hormone Neurons Show a Different Migratory Activity *in Vitro**

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ABSTRACT

The development of two cell lines (GT1 and GN) of immortalized LHRH neurons has allowed an accurate study of the mechanisms controlling the synthesis and the secretion of LHRH. These cell lines, obtained in mice by genetic targeted tumorigenesis, retain many of the phenotypic characteristics of LHRH neurons. Of interest, GT1 cells derive from an hypothalamic tumor, whereas GN cells were obtained from a tumor localized in the olfactory bulb. The different origin of these cell lines lead to hypothesize that they might represent hypothalamic postmigratory neurons (GT1 cells), or LHRH neurons blocked at an early stage of their migration (GN cells). Using different experimental procedures, we found that the two cell subclones GT1–7 and GN11 express a different morphology and migratory behavior *in vitro*. In particular, we found that GN11 cells, but not GT1–7 cells, show the morphological shape of migrating neurons. When analyzing the spontaneous motility we found that only GN11 cells express a high capacity of migrating in a matrix of collagen gel. Moreover, in a chemomigratory assay GN11 cells did show a significant response to the chemotactic stimulus represented by the FBS. On the contrary, GT1–7 cells show very low spontaneous motility and appear insensitive to the FBS stimulus. These results suggest that the simultaneous use of the GT1–7/GN11 cells may represent an experimental tool for screening the factors possibly involved in the control of the migratory processes of LHRH neurons in normal and in pathological conditions, such as those due to their impaired migration, like it happens in Kallmann's syndrome. (*Endocrinology* **141:** 2105–2112, 2000)

HYPOTHALAMIC LH-releasing hormone (LHRH)-producing neurons originate outside the central nervous system. They arise in the olfactory placode and during their development they migrate, along the terminalis and vomeronasal nerves, into the developing forebrain to reach their final destination in the septum and in the preoptic area of the hypothalamus (1, 2). At the end of their migration, LHRH neurons project axons to the median eminence to make contacts with the pituitary portal vessels. This migratory pattern has been observed in man and in several other species and it is fundamental for the development of normal reproductive functions (3). In fact, an impaired migration of the LHRH neurons is a likely pathogenic factor of the hypogonadotropic hypogonadism occurring in patients affected by the Kallmann's syndrome (4, 5).

The LHRH system is characterized by a peculiar anatomical location; it is composed of about a few hundred neurons distributed as clusters that are present in many areas extending from the preoptic to the anterior hypothalamic regions (6). This organization makes it difficult to determine, by *in vivo* or by *ex vivo* experiments, whether the different factors influencing LHRH release exert their effects directly on the LHRH-synthesizing neurons, or if they act via the activation and/or the inhibition of other neuronal systems impinging on these neurons (7).

The study of the molecular biology of the LHRH neuronal system was made easier by the availability of immortalized LHRH-secreting neurons. Two different cell lines, the GT1 cells (with the GT1–1, -3, and -7 subclones) (8) and the GN cells (with the GN10, GN11, and NLT subclones) (9), were obtained by genetically targeted tumorigenesis of LHRH neurons in mice. Biochemical and functional studies have shown that these cells retain many characteristics of hypothalamic LHRH-secreting neurons (10, 11) thus making them an invaluable tool for the study of the regulation of LHRH gene expression, and of processing and release of the peptide. Actually, several results obtained on immortalized LHRH neurons have been recently confirmed by studies performed *in vivo* and *in situ* on normal hypothalamic LHRH neurons (12–14).

GT1 and GN cell lines were obtained by dispersion of brain tumors developed in transgenic mice expressing an hybrid gene; this was formed by the coding region of the SV40 large T-antigen oncogene fused with a portion of the promoter region of rat (GT1 cells) and human (GN cells) LHRH gene. Of interest, GT1 cells have been derived from a transgenic mouse that developed an hypothalamic tumor, *i.e.* they derive from LHRH neurons that have already migrated to reach their final destination (8); on the contrary, GN cells were obtained from a mouse having a tumor in the olfactory bulb, *i.e.* they originate from LHRH neurons blocked at an early stage of their migration (9). Because of their different site of origin, it has been hypothesized that GT1 and GN cells may be representative, respectively, of postmigratory and migra-

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tory LHRH neurons (15). Surprisingly, studies describing a direct comparison of the biochemical characteristics— which probably differentiate GT1 from GN cells—are still at a very preliminary phase (10, 15–18); moreover, no study has been dedicated so far to analyzing possible differential migratory activities of the two cell lines.

The experiments described here following have been designed to compare directly the migratory activity *in vitro* of GT1–7 and GN11 cells.

Materials and Methods

Cell cultures

GT1–7 cells, provided by Dr. R. I. Weiner (San Francisco, CA) and GN11 cells, provided by Dr. S. Radovick (Boston, MA), were grown on uncoated plastic Petri dishes in complete culture medium (DMEM, Biochrom KG, Berlin, Germany) supplemented with 10% FBS (Life Technologies, Inc., Grand Island, NY) as already described (16).

Cell aggregates and collagen gel assay

Collagen gel is a widely used procedures to study normal as well as transformed cells. This matrix has been found to allow optimal physiological culture conditions for many tissue fragments and normal isolated cells and to study cell migration (19–26). Of interest, it has been found that neuronal precursor cells reorganize neural tube-like structures when cultured in collagen-gel matrix (24). Cell aggregates were prepared by the hanging drop technique (25); subconfluent cells were collected by trypsinization, resuspended in complete culture medium and seeded in 20-μl drops (200,000 cells for both cell lines) on the lid of a culture dish; the lid was then placed on a dish filled with 2 ml of culture medium and incubated at 37 C for 48 h.

Rat tail collagen stock solution was prepared as described (22, 26). Collagen gel was obtained by addition of 10% (vol/vol) of 10× concentrated DMEM (without phenol red) and 0.8 M bicarbonate to an aliquot of collagen stock solution. This solution (20 μl) was pipetted onto the bottom of a well of a 24-well culture dish, and left to set at room temperature. Cell aggregates were transferred over the cushion and then overlaid with additional 20 μl of collagen. As the overlaid collagen was set, it was covered with 400 μl DMEM (supplemented with 1% FBS) and transferred to the cell culture incubator. The aggregates were observed daily under a light microscope; at the end of the incubation time phase-contrast pictures of the aggregates were taken, the viability of the cells forming the aggregates was then tested by incubation for 30 min with a solution of a diphenyl-tetrazolium salt (MTT, Sigma, St. Louis, MO) (1 mg/ml in phenol red-free culture medium); the formation of blue formazan crystals was taken as indicative of healthy metabolically active cells (27). Then, the aggregates were fixed in 4% paraformaldehyde. Some of the aggregates not used for MTT analysis were then stained with a 0.5% cresyl violet solution or embedded in paraffin and sectioned for the cytopathological analysis.

Cultures on glass fibers

Glass fibers, derived from commercial glass wool (5 mm length), were prepared as described (28) and precoated with poly-L-lysine. After a series of washes with PBS, the fibers were transferred over an uncoated glass coverslip (12 mm diameter) put in the bottom of a culture plate. GT1–7 and GN11 cells (10,000 cells/well) resuspended in complete culture medium were then seeded over the coated glass fibers. Twenty-four hours later, the cells were directly observed under phase contrast microscope, then fixed for 10 min in 4% paraformaldehyde and stained with phalloidine-FITC (Sigma) as described (29).

Chemomigration assay

The assay was performed using a 48-well Boyden's microchemotaxis chamber according to manufacturer's instructions (Neuroprobe, Cabin John, M.D.). Briefly, the cells grown in complete medium until subconfluence were harvested, and the suspension (10^5 cells/50 μl of serum-free DMEM for both cell lines) was placed in the open-bottom wells of the upper compartment. Each pair of wells were separated by a polyvinylpyrrolidone-free polycarbonate porous membrane (8-μm pores) precoated with gelatin (0.2 mg/ml in PBS). For chemotaxis (CT; the directed migration of cells toward regions of higher concentration of chemotactic factors) experiments, the chemoattractants (FBS or Sato's chemical-defined medium: DMEM 100 ml, 500 μg insulin, 10 mg transferrin, 1.6 mg putrescine, 2 μl of 20 nM solution selenite) was placed into the wells of the lower compartment of the chamber thus mimicking the diffusible gradient of molecules that cells might encounter *in vivo*. Chemokinesis (stimulation of increased random cell motility) was distinguished from chemotaxis by placing the same concentration of chemoattractant in both the upper and the lower wells of the Boyden's chamber, thereby eliminating the chemical gradient. The chamber was then kept for 3 h in the cell culture incubator. After incubation, the cells migrated through the pores, and adherent to the underside of the membrane, were fixed and stained according with the Diff-Quick stain kit (Biomap, Italy) and mounted onto glass slides. For quantitative analysis, the cells were observed using an oil immersion 100× objective on a light microscope. Six random objective fields of stained cells were counted for each well and the mean number of migrating cells/mm^2 was calculated. The number of migrated cells, obtained from at least 10 independent wells for each group, were compared by ANOVA and Dunnett or Scheffé tests.

Results

Morphology and growth characteristics

GT1–7 cells appear as neuronal-like polygonal cells, with small perikarya, which are interconnected through neuritic processes (Fig. 1A). They grow mainly in colonies, which

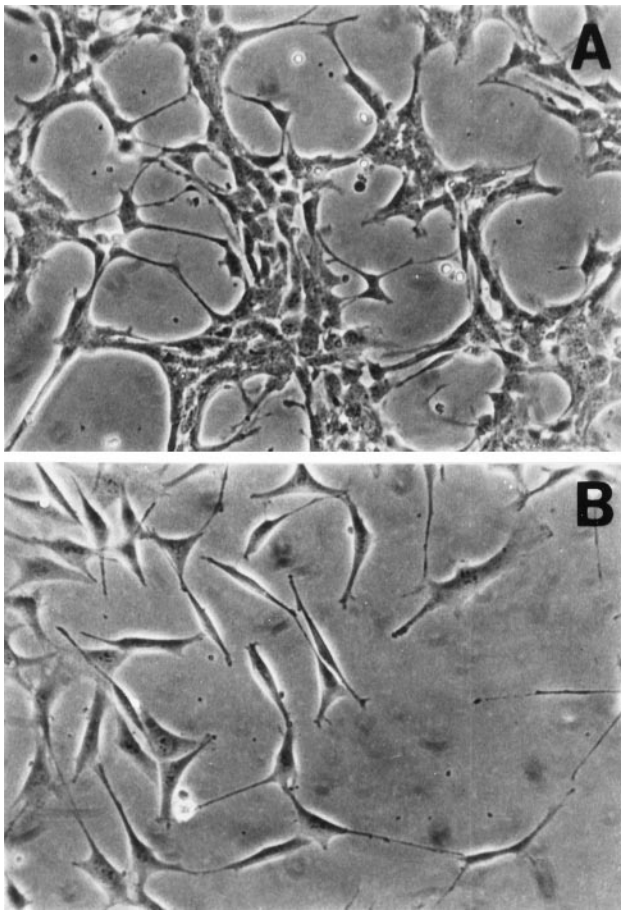

FIG. 1. Phase-contrast photomicrographs of GT1–7 (A) and GN11 (B) cells growing on plastic dishes (200×).

establish connections during the time of culture. GT1–7 cells reach the confluence through an increase of the size of the colonies, mainly due to the peripheral apposition of newly duplicated cells.

In contrast to GT1–7 cells, GN11 cells do not form clusters (Fig. 1B), but show an homogeneous growth on the whole surface of the culture dishes. They appear bigger than the GT1–7 cells (their size is almost double), and show generally a bipolar morphology, with a major proportion of fusiform cells bearing one or more neuritic processes (Fig. 1B).

Migration of immortalized LHRH neurons from cell aggregates

The collagen gel assay is a generally accepted procedure to analyze in a three-dimensional matrix cell migration, as well as axonal sprouting from neuronal cells in aggregates or tissue explants (19–26). The ability of GT1–7 and GN11 cells to migrate spontaneously from cell aggregates into a matrix of collagen gel in response to FBS, used as a general stimulus, has been therefore analyzed. First of all, we found that both cell lines spontaneously form cell aggregates when prepared by the hanging-drop technique, suggesting than these cell lines express efficient cell-cell interaction. The two types of aggregates (obtained by a suspension of the same number of cells) have been then incubated for 48 h in collagen gel either in the presence of 1% FBS (a condition of low proliferative activity) (Table 1) or in the absence of FBS.

TABLE 1. Percent variation of the number of GT1-7 and GN11 cells exposed for 48 h to different concentrations of FBS during the exponential growth

Medium	GN11	GT1-7
DMEM	100 ± 8	100 ± 5
DMEM+1% FBS	115 ± 13	124 ± 15
DMEM+10% FBS	158 ± 18[a]	193 ± 9[a]

The cells were seeded on plastic dishes, in complete culture medium, at a density near to 30% confluence and left to adhere for 24 h before incubation with the different media. Cell number was determined by the trypan exclusion method using the Burker's hemocytometer. Values are expressed as mean ± SEM; [a] $P < 0.05$ significant *vs.* DMEM.

In these culture conditions, the two cell lines show a markedly different behavior. In particular, GT1–7 cells appear to be completely unable to move from their aggregate form when incubated for 48 h in the presence of FBS (Fig. 2A); a similar picture was observed for aggregates of GT1–7 cells also when cultured in the absence of serum (data not shown). On the contrary, a large number of GN11 cells, when cultured with 1% FBS, actively leave the aggregate to migrate into the collagen matrix (Fig. 2B). The migration of GN11 cells into the collagen matrix was negligible in the absence of FBS, suggesting a role of serum factors in such an effect (Fig. 2C). The positive reaction of all the cell aggregates to the MTT test (Fig. 2, D–F) is clearly indicative of the good viability of these cells in all the experimental conditions. This was also confirmed by results of the experiments in which the cells, obtained by enzymatic dispersion of the aggregates cultured for 48 h in collagen gel, showed a further normal growth rate when seeded on plastic dishes (data not shown). Representative aggregates incubated for 48 h in collagen gel were also paraffin embedded, sectioned and stained with a standard hematoxylin-eosin procedure. The cytopathological analysis of these preparations showed that the cells are uniformly distributed into the aggregates, with no picnotic nuclei or necrotic cells (Fig. 3). All of these evidences indicate that the lack of migratory activity observed in GT1–7 cells, and in GN11 cell aggregates tested in FBS-free medium, is not due to a decreased cell viability.

Additional data have shown that the migratory event of

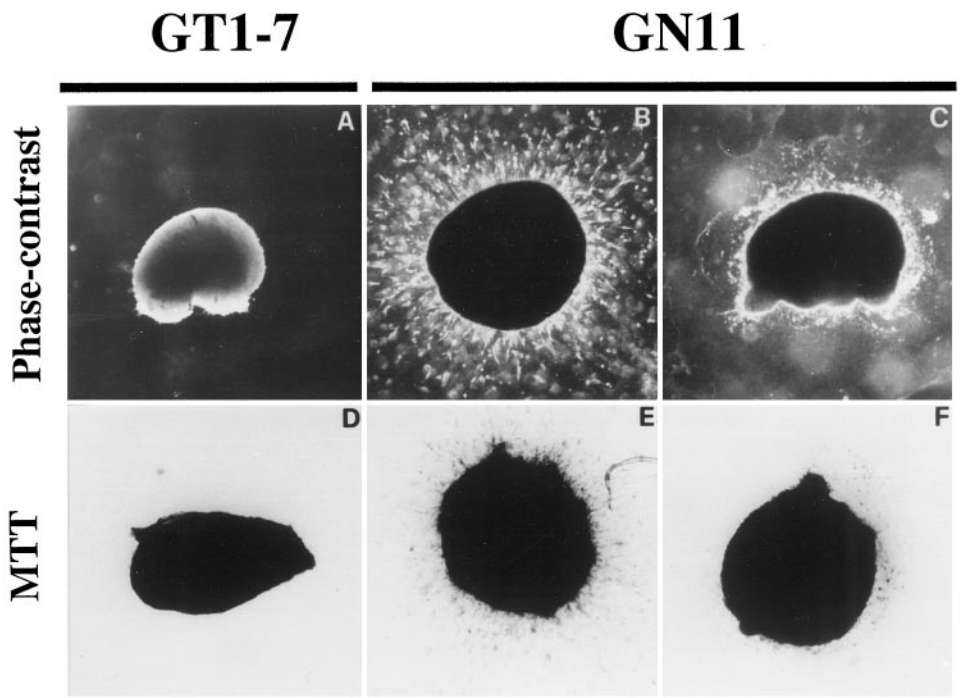

FIG. 2. Migration of GN11 cells, but not of GT1–7 cells, from a cell aggregate into a matrix of collagen gel. A and B, Phase-contrast photomicrographs of GT1–7 and GN11 cell aggregates, respectively, cultured for 48 h in collagen gel in the presence of 1% FBS; C, GN11 cell aggregates cultured for 48 h in the absence of FBS. D–F, Bright-field images of aggregates cultured in parallel to those shown respectively in A–C and tested for cell viability by MTT assay. The dark staining is indicative of viable cells (see *Materials and Methods*) (30×).

GN11 is not dependent on the initial size of the aggregate. This is indicated in Fig. 4A, in which GN11 aggregates formed by different number of cells were incubated for 48 h in collagen gel.

Generally, GN11 cells in collagen gel appears to migrate radially from the aggregate and cords of migrating cells are frequently observed (Fig. 4B), suggesting the formation of close cellular association during the migratory movement. The microscopic observation at higher magnification also shows that GT1–7 cells do not leave their aggregates although they may extend short neurites into the collagen gel (Fig. 5A). On the contrary, a large number of GN11 cells penetrate the matrix assuming a bipolar-fusiform morphology (Fig. 5B).

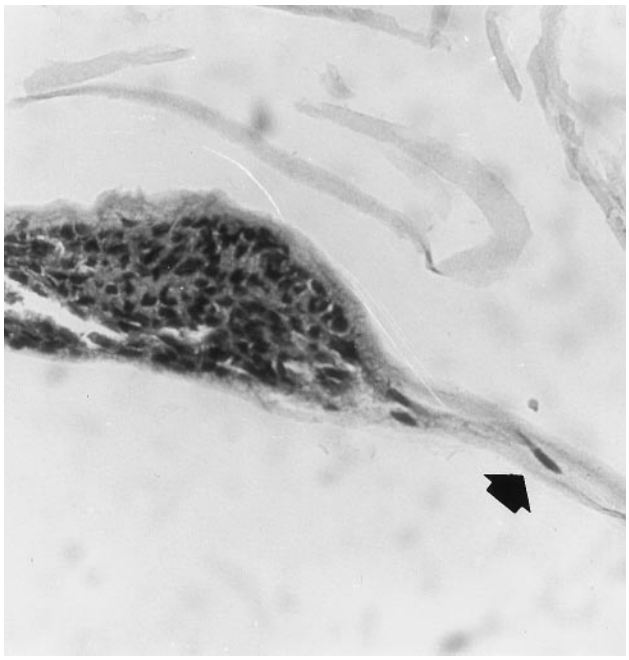

FIG. 3. Photomicrograph of a section of one representative cell aggregate (GN11 cells in 1% FBS) cultured for 48 h in collagen gel matrix. Similar pictures were obtained either from GT1–7 cell aggregates cultured with 1% FBS or from the aggregates of the two cell lines cultured in the absence of FBS. A GN11 cell migrating from the aggregate is indicated by the *arrow* (hematoxylin-eosin staining, 260×).

Morphology of immortalized LHRH neurons on glass fibers

To get further information on the cytology of the two cell lines of immortalized LHRH neurons, experiments have been then performed to analyze the shape assumed by GT1–7 and GN11 cells cultured on poly-lysine coated glass fibers. The glass fibers mimic the geometry of the fascicles of glial or neuronal processes possibly present along the migratory pathways (28) and provide the opportunity to examine the profile of the cells. First of all, it has been found that only a few GT1–7 cells adhere to coated glass fibers, and that adherent cells assume an atypical spherical shape (Fig. 6A). On the contrary, a large number of GN11 cells adhere to poly-lysine coated glass fibers, and, in contrast to GT1–7 cells, show an asymmetric shape with a prominent leading process (Fig. 6, B and C). Again, cords of associated GN11 cells along the same glass fiber have been observed (Fig. 6D).

Chemomigration assay

To get quantitative results on the migratory activity of GN11 cells, a series of chemomigration assays using Boyden's chamber has been performed. This method provide a sensitive measure of the cellular response to specific chemotropic signals. In the absence of information on the existence of specific chemoattractants for the two cell lines used, the experiments have been performed using FBS as a general

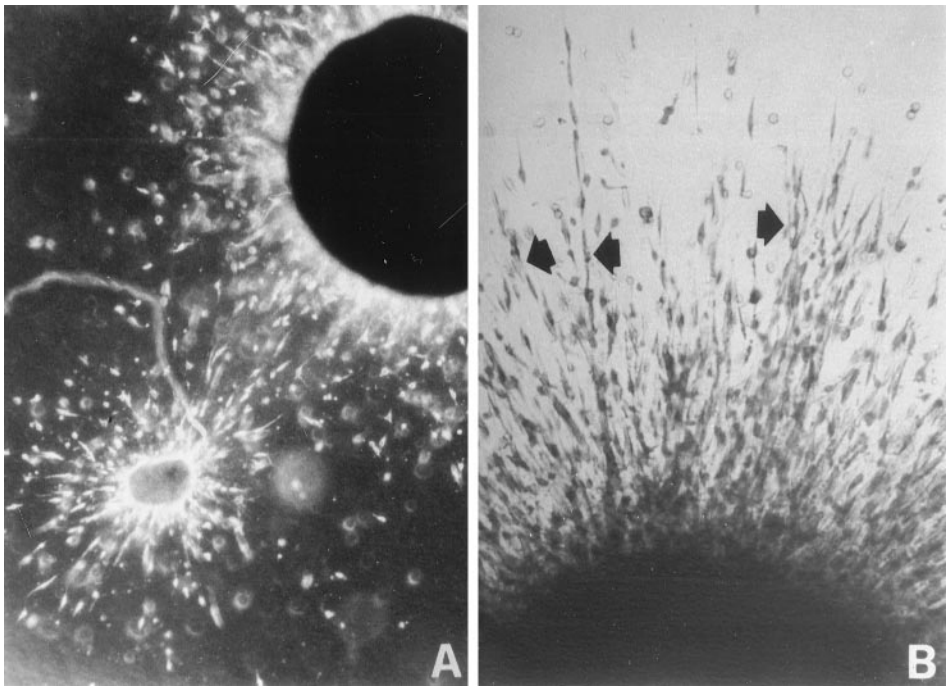

FIG. 4. A, Phase-contrast photomicrograph of two GN11 cell aggregates of different size cultured for 48 h in collagen gel matrix with 1% FBS containing medium (45×); B, bright field picture of GN11 cell aggregate showing cords of migrating cells (*arrows*) (Cresyl violet staining, B, 130×).

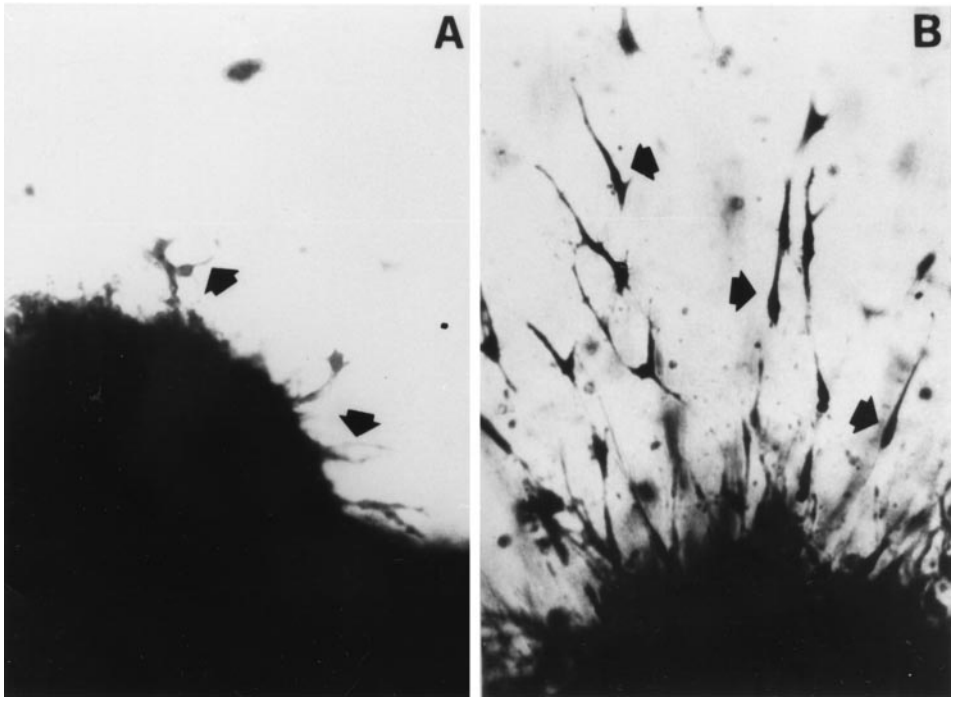

FIG. 5. GT1–7 (A) and GN11 (B) cell aggregates cultured for 48 h in collagen gel in the presence of 1% FBS. Small axons extending from the GT1–7 aggregate are indicated (A, *arrows*). GN11 cell migrating into the collagen matrix showing a clear fusiform morphology (B, *arrows*) (Cresyl violet staining, 220×).

chemotactic stimulus. First of all, the results show once more that GT1–7 cells show a negligible spontaneous motility in the absence of FBS; in addition, these cells do not show any chemotactic response to increasing concentrations of FBS added in the lower compartment of the Boyden's chamber; chemokinetic responses were also absent when 5% FBS was added in both compartments of the chamber (Fig. 7A).

GN11 cells also show a very low spontaneous motility in the absence of FBS; however, FBS was highly efficient in stimulating in a concentration-dependent manner the chemotaxis of these cells (Fig. 7B). In particular, a highly significant chemotactic response is already evident at 0.1% FBS concentration; a further and maximal increase of the response is evident at 0.5% FBS. In addition, Fig. 7B shows the failure of a serum-free chemical-defined medium (Sato's medium; see *Materials and Methods*), containing only insulin as a growth factor and widely used for primary cultures of neuronal cells, to exert any chemotactic effect of GN11 cells; this result indicates that migration is induced by factors, present in FBS, other than those present in the defined medium. Other data have shown that a 5% serum concentration of FBS, added on both compartments of the Boyden's chamber, induced chemokinesis in GN11 cells; however, the induction of this random cell motility is significantly less efficient than that induced by the chemotactic stimulus exerted by the same concentration of FBS.

Finally, to verify a possible link between the chemomigratory and the proliferative activities of GN11 cells, we have analyzed the chemotactic response, induced by increasing concentration of FBS, after 24 h of serum deprivation (a procedure known to slow down cell duplication and to synchronize a large proportion of cells in the G1 phase of the cell cycle) (30). The results obtained indicate that serum-deprived cells are still responsive to the chemotactic FBS stimulus (Fig. 8), which induced an effect not statistically different from that observed in cells placed in FBS-supplemented culture medium until the time of the assay (see Fig. 7B).

Discussion

The results of the experiments here presented show that GN11 cells, but not GT1–7 cells, have the competence to migrate in appropriate tests *in vitro*.

As described in the results section, the ability of migrating into a collagen gel matrix of the two cell lines of immortalized LHRH-secreting neurons was first evaluated. It has been found that GN11 but not GT1–7 cells show an intense migration into the collagen gel matrix from a cell aggregate; migrating cells show a bipolar fusiform morphology and leave the aggregate in cords of associated cells with a radial orientation; this suggests the presence of a coordinated rather than of a random motility. The results also show that the migratory activity of GN11 cells depends on the presence of FBS; however, the MTT assay and the cytopathological analysis of the cell aggregates exclude the possibility of a loss of viability in non migrating cells.

Considering that migration into the collagen matrix might be linked to the invasiveness typical of tumor derived cells (31) and that this might have been retained by GN11 cells but not by GT1–7 cells, we have verified the migratory activity of the two immortalized LHRH neurons using other two different methodological approaches. We first analyzed the shape that GT1–7 and GN11 cells would take when made to adhere to poly-lysine-coated glass fibers. We found that GN11 cells assume a typical profile of migratory neurons (bipolar shape and the extension of a leading process) (28, 32), whereas GT1–7 cells do not.

Finally, we performed a sensitive chemomigration assay using Boyden's chamber to evaluate the chemotaxis and the

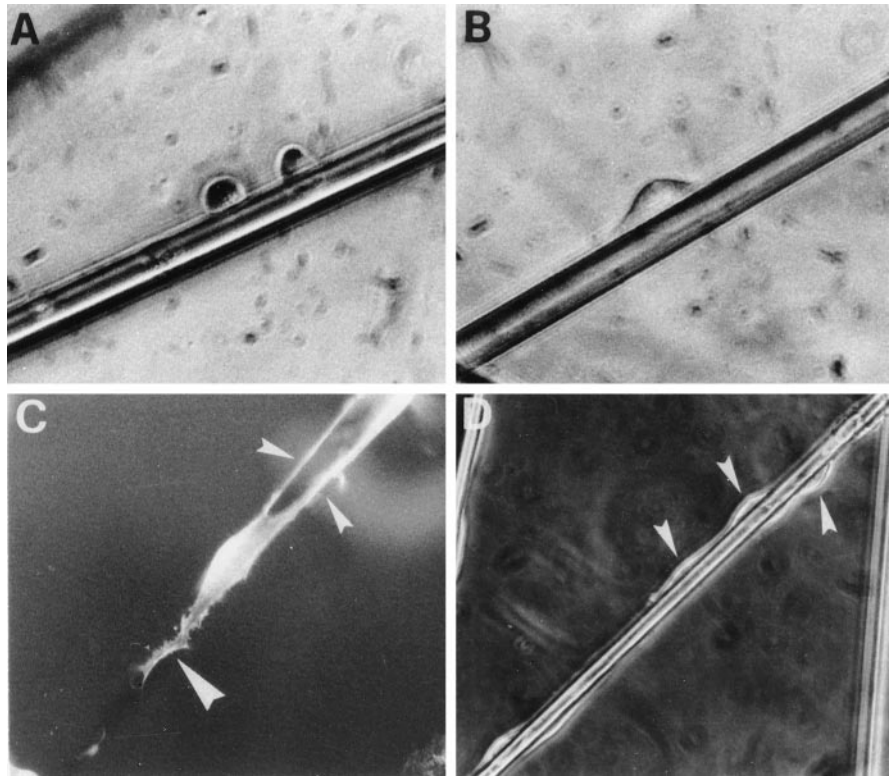

FIG. 6. Cytology of GT1–7 (A) and GN11 (B, C and D) cells made to adhere to poly-lysine coated glass fibers. GN11 cells show an asymmetric profile (B) with leading process (C; *large arrowhead*); *small arrowheads* indicate the leading processes of other cells present on the same fiber and also evident in panel D. (A, B, and D, phase-contrast; C, phalloidine-FITC stained cells; A–C, 530×; D, 200×).

chemokinesis induced by FBS (see *Materials and Methods*). The results showed that GN11 cells respond, in a dose-related fashion, to the chemotactic stimulus provided by FBS, even if this was used at very low concentrations. On the contrary, GT1–7 cells did not show any motility in the chemomigratory tests performed in the present study; this is consistent with the results obtained in the collagen gel assay. It is reported in the literature that the sensitivity of cells to chemical gradients may generally discriminate differences as low as 2% of the concentration of the chemoattractants (33). Of interest, the present study shows that a 0.1% concentration of serum was sufficient to induce a large chemomigratory response in GN11 cells. The present data also show that FBS was significantly more efficient in stimulating chemotaxis than chemokinesis; this indicates the presence in FBS of specific chemotactic signals in addition to general inductors of cell motility. The chemotaxis assay has been designed (see *Materials and Methods*) to present a gradient of a putative chemoattractant to small populations of cells (those near to the gradient source), whereas chemokinesis tests involve the exposure of the whole population of cells to a uniform concentration of a chemoattractant. Therefore, the evidence here presented of a chemotactic response higher than the chemokinetic one is indicative of a polarized sensitivity of GN11 cells to a chemical gradient, a typical characteristic of migratory cells (33).

The observation here reported that the migratory activity of GN11 cells depends on the presence of FBS might suggest that these cells respond to the general trophic effect provided by serum on their survival and proliferation. However, several of our experimental observations rule out a link between the motility and the proliferative activity of GN11 cells. In fact: 1) GN11 cells, like GT1–7 cells, proliferate in response to serum in normal culture condition; however, only GN11 cells show a migratory activity, while GT1–7 cells do not migrate; 2) the serum concentration (1%) used in collagen gel assay allows very low proliferative activity of GN11 cells; 3) low concentration of serum (0.1%) and short times of incubation (3 h) are sufficient to promote a potent chemotactic stimulus; 4) the preincubation for 24 h in serum-deprived medium does not change the chemotactic response of GN11 cells to the FBS stimulus. Anyway, migration and proliferation may occur simultaneously. In fact, an unique characteristic of neuronal olfactory precursors involves their ability to undergo active cell division during migration (34); this behavior has been also proposed for some populations of LHRH neurons (35).

The unresponsiveness of GN11 cells to Sato's medium in the chemomigration experiments indicates that, despite the known ability of this medium to support survival of neuronal cells in culture, the factors included in its formulation, and possibly present also in the serum (*i.e.* insulin), do not exert chemotactic effects.

The chemomigratory response of GN11 cells to a general stimulus, like FBS, is not surprising; obviously, neuronal cells *in vivo* are continuously exposed to blood and serum which may support their motility. Of interest, the migratory route of LHRH neurons is in close apposition with blood vessels suggesting that serum factors might play distinct roles on the physiology of these neurons. Future experiments will be needed to identify the factors in FBS involved in maintaining the motility of GN11 cells. On the other hand, growing evidence suggests that neuronal migration may be considered a programmed spontaneous event occurring during devel-

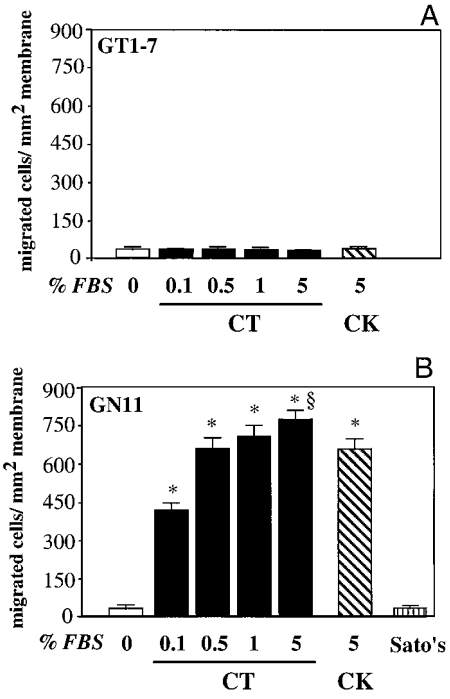

FIG. 7. Chemotactic (CT) and chemokinetic (CK) responses of GT1–7 (A) and GN11 (B) cells. The chemomigration assay was performed using Boyden's chamber. Chemotaxis was measured in the absence or in the presence of increasing concentrations of FBS or of chemical-defined medium (Sato) as chemoattractants. Chemokinesis was analyzed in the presence of 5% FBS. Results are expressed as mean ± SEM. *, Significant $P < 0.01$ *vs.* control (0% FBS); §, significant $P < 0.05$ *vs.* CK.

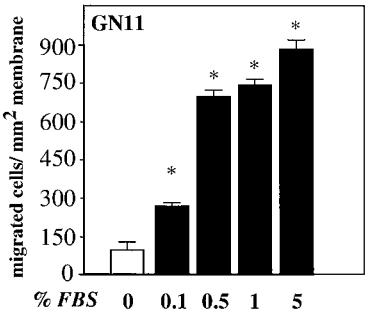

FIG. 8. Chemotactic response of GN11 cells cultured for 24 h in serum-deprived medium before the chemomigration assay. This was performed using Boyden's chamber in the absence or in the presence of increasing concentrations of FBS. Results are expressed as mean ± SEM. *, Significant $P < 0.01$ *vs.* control (0% FBS).

opment; specific glial- and neuronal-derived signals would exert a role in directing the migratory movement until stop signals determine the final location of migrating neurons (36, 37). In support of this line of thinking, one may refer to the *in vitro* migration of LHRH neurons out of tissue explants (obtained from olfactory placode or from regions of their migratory pathway) in the absence of specific chemoattractant in the culture medium (38–40).

The absence of a response of GT1–7 cells to FBS may indicate that these cells have lost the responsiveness to a specific serum factor(s) or, alternatively, that they may have developed a high cell-to-cell adhesion which may block their motility. In agreement with the results here reported, it has been found that GT1 cells, implanted into the mouse brain, show a poor migratory activity although they may occasionally send their axons toward the median eminence (41).

The possibility that the migratory activity of GN11 cells might be the result of the reappearance of cell motility due to their tumoral transformation has also been considered. It should be noted that both GT1–7 and GN11 cell lines derive from a tumor originated by LHRH-expressing neurons and induced by the same oncogene (SV40-Tag). Should the hypothesis be correct, one could expect also GT1–7 cells to express some sort of motility in the various tests performed. Moreover, SV40-transformed cells were generally found to be minimally invasive (42), and no metastatic diffusion was described in mice carrying the tumors which gave origin to the GN or to the GT1 cells or other tumors induced by SV40-Tag oncogene (8, 9, 43). These considerations bring to the conclusion that GN11 cells might have maintained the proper migratory activity of migrating neurons. In line with this suggestion, preliminary experiments performed in the authors' laboratory show that GN11 cells stably express in culture the characteristics of immature neurons (like the expression of nestin protein and a low electrical activity) (Pimpinelli, F., B. Rosati, E. Wanke, L. Molteni, and R. Maggi, in preparation); this observation fits well with their migratory activity.

According to our data, the expression of the oncogene seems to have frozen the maturation of the two types of neurons at two different stages of their development, characterized, respectively, by the presence (GN11) or by the absence (GT1) of migratory capability. This hypothesis is supported by the recent findings that immortalized pituitary cells, obtained by Tag-induced targeted tumorigenesis, maintain the phenotypic markers of the developmental stage at which the activation of the hybrid oncogenic transgene has occurred (43, 44). Of interest, Tag-transformed cells (45, 46) and neurons (47) have been found to recover cell maturation and differentiation after inactivation of the oncogene.

Based on the assumption that the GT1- and GN-derived cell lines are respectively representative of postmigratory and migratory LHRH neurons, Fang *et al.* (15) compared GT1–7 and GN10 cells, by differential display-PCR, to search novel factors possibly involved in the control of LHRH neuronal migration. They found that these cell lines differ in the transcription of several genes; one of the gene found to be selectively activated in GN10 cells encodes for the putative tyrosine kinase receptor Ark. It has been subsequently shown (18) that the activation of this receptor may be involved in protecting GN10 cells from programmed cell death; on the basis of this finding, these authors have therefore proposed a role of Ark signaling in the protection of LHRH neurons from apoptosis during neuronal migration.

In conclusion, the data reported in the present paper clearly indicate that GN11 and GT1–7 cells show a different behavior in their migratory activity *in vitro*, suggesting that these immortalized LHRH neurons, besides their well accepted use for the study of the synthesis and release of the decapeptide, may also represent a useful model to screen some of the mechanisms affecting neuronal migration. The

chemomigration assay is a kind of study that may be only performed *in vitro* and on a homogeneous population of neurons. Primary cultures of migrating LHRH neurons from the olfactory placode, which are useful for biochemical studies (38–40), are not pure enough to be used for quantitative chemomigration assays; moreover, they undergo cell differentiation during long-term cultures (39).

The information obtained using the model here presented will now allow to better design *in vivo* experiments in the effort of clarifying the physiological mechanisms involved in the migration of LHRH neurons as well as some pathogenic aspects of hypogonadotropic hypogonadism, so far described as idiopathic (IHH), and particularly of those associated to Kallmann's disease.

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