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Short Communication

Expression profiling of genes encoding glutamate and GABA receptor subunits in three immortalized GnRH cell lines

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ABSTRACT

Gonadotropin-releasing hormone (GnRH) plays a central role in regulating development and function of the reproductive axis, and its secretion is known to be influenced by glutamate and GABA. In the present study, we used gene microarrays and RT-PCR to compare the expression profiles of glutamate and GABA receptor subunits in three immortalized GnRH cell lines: GT1-1, GT1-7, and Gn10. All of these cell lines expressed the AMPA glutamate receptor subunit genes GluR2 and GluR4, but only the GT1-1 and GT1-7 cells expressed the kainate glutamate receptor subunit gene KA2. Additionally, GluRδ2, a subunit that can form heteromeric receptors with kainate and AMPA subunits, was present in GT1-1 and Gn10 cells but not in GT1-7 cells. Genes encoding the $GABA_A$ receptor α3, β2, β3, ε, and π subunits, as well as the $GABA_B$ receptor 1 subunit, were evident in all three cell lines. However, the gene encoding the expression of $GABA_A$ receptor γ subunit was noticeably absent. Taken together, these data demonstrate comprehensive screening of neurotransmitter receptor genes in a controlled neuronal culture system, and reveal novel features.

Gonadotropin-releasing hormone (GnRH) is the primary neuropeptide regulating development and function of the mammalian reproductive axis (Jennes and Conn, 2002). GnRH neurons have a diffuse distribution pattern within the forebrain, which makes it difficult to study their afferent neural connections. In contrast, immortalized GnRH cell lines such as GT1-1, GT1-7 and Gn10 (Mellon et al., 1990; Radovick et al., 1991) can be propagated on a large scale in vitro, and so offer a pragmatic approach to studying the molecular biology of GnRH neurons in a controlled environment.

Glutamate and γ-aminobutyric acid (GABA) are the primary neurotransmitters modulating GnRH release (Leranth et al., 1985; Urbanski and Ojeda, 1990; Hales et al., 1994; Martinez de la Escalera et al., 1994; Spergel et al., 1995; Urbanski, 1996; Urbanski et al., 1996; Brann and Mahesh, 1997; Spergel et al., 1999; Gore, 2001; Defazio et al., 2002), and attempts have already been made to characterize the composition of glutamate and GABA receptor subunits in GnRH neurons and also in immortalized GT1-7 cells (Hales et al., 1992; Favit et al., 1993; Petersen et al., 1993; Urbanski et al., 1994; Eyigor and Jennes, 1996; Sortino et al., 1996; Jung et al., 1998; Mahesh et al., 1999; Pape et al., 2001). However, because these previous results are incomplete, we used high throughput genetic screening to more comprehensively characterize glutamate and GABA receptor gene expression in GnRH cells that had been immortalized early (Gn10) or late (GT1-1 and GT1-7) during embryonic development. Preliminary findings have been published in abstract form (Garyfallou and Urbanski, 2004).

The cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2/95% air in DMEM (Sigma-Aldrich, St. Louis, MO) containing 4.5 mg glucose ml^{-1}, 0.6 mg L-glutamine ml^{-1}, 100 U

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penicillin ml^{-1}, 100 g streptomycin ml^{-1} (Sigma-Aldrich), supplemented with 10% fetal bovine serum (HyClone Laboratories, Inc. Logan, UT), as described (Mellon et al., 1990). Total RNA was isolated using RNeasy Midi Kit (Qiagen Inc., Valencia, CA). RNA probe synthesis, hybridization and array scanning were carried out according to the Affymetrix GeneChip Expression Analysis Technical Manual (Affymetrix Inc., Santa Clara, CA). Duplicate aliquots from each cell line were hybridized to a total of six mouse microarrays (Affymetrix MOE 430A). Statistical algorithms from the Affymetrix MicroArraySuite software (MAS 5.0) were used to generate signal metrics. The parameters α_1 and α_2, which set the point at which the probeset is called present, marginally present, or undetectable, were set to 0.1 and 0.15, respectively. GenBank accession numbers were used for identification and annotation of genes represented on the array.

In addition, total RNA extracts (2 μg) from each cell line were converted to cDNA using the Omniscript Reverse Transcriptase kit (Qiagen Inc.), and PCR was performed using the HotStarTaq PCR kit (Qiagen Inc.). The essential sequence-specific oligonucleotides were synthesized by Invitrogen (Carlsbad, CA) (Table 1). Thermocycler parameters were: 95 °C for 15 min, denaturation 94 °C for 1 min, annealing 60 °C (annealing temperature) for 1 min, and 72 °C for 1 min. After 30 cycles and a final elongation step at 72 °C for 10 min, 10 ml of the PCR products were separated electrophoretically on a 1.8% agarose gel containing ethidium bromide and visualized using an u.v. transiluminator. Reactions without cDNA served as negative controls. Because the mouse GnRH and GnRH-associated peptide (GnRH-GAP) target sequence is not in the MOE 430A GeneChip microarray, we also performed RT-PCR to confirm that each of the three cell lines express the gene encoding GnRH-GAP. Notably, GnRH-GAP gene expression was considerably lower in Gn10 cells than in GT1-1 or GT1-7 cells (Fig. 1); the former represent GnRH cells from a pre-migratory stage of development, whereas the latter two represent post-migratory GnRH cells (Mellon et al., 1990; Radovick et al., 1991).

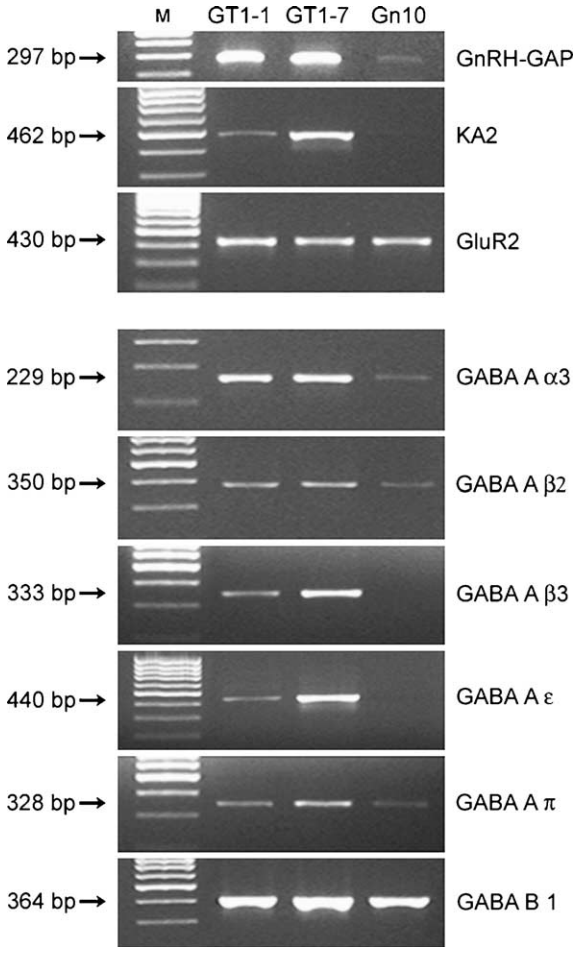

Fig. 1 – Differential expression of genes encoding glutamate and GABA receptors subunits in three immortalized GnRH cell lines, revealed by RT-PCR amplification. A molecular weight marker (M) was loaded in the first lane of each gel. In all cases, the product size obtained matched the predicted size for the primers used.

Table 1 – Sequence-specific oligonucleotides used for PCR

Gene	Sequence 5′–3′	Orientation
GnRH-GAP	AACTGATGGCCGGCATTCTACTGC	F
	CATCTTCTTCTGCCTGGCTTCCTC	R
KA2	AGCTGACCGAGCACGAATGACCG	F
	AGGGGCCCAAGAGACCCTCTCC	R
GluR2	GTATGCCTACTTGCTGGAGTCC	F
	GGGAATTCTGCGAGGAAGATGG	R
Gabra3	TCTGGCCAAGGATACTGAGTTCTC	F
	TTTGCGGATCATGCCCTTGATAGC	R
Gabrb2	GCTCTGGAGCGACATGTGGCAC	F
	CCGCGAGGACCTTAGTGTATTCC	R
Gabrb3	ATCGACATGTACCTGATGGGCTGC	F
	CTTGTGCGGGATGCTTCTGTCTCC	R
Gabre	TCCCTCGTGTCTCCTATCTCACGG	F
	CACCCGCGAATAGTTATCCAGACG	R
Gabrp	GTGTAGAAGCCCTAGTGTGACC	F
	CCACCAATGAATTGACGCAAACCC	R
Gabbr1	ACACCACAGTGTGAAAGGGATGCTG	F
	GGGATTACAGTTGTCTGCCTAGCTG	R

The use of GeneChip microarrays in the present study enabled us to examine the relative expression of 35 of the known genes encoding various glutamate and GABA receptor subunits (Table 2). In the case of glutamate receptors, previous histochemical studies showed that KA2 is coexpressed with GluR5 in 40–50% of rat GnRH neurons (Eyigor and Jennes, 1996), implying the presence of functional KA receptors. Also, Northern analysis showed that GT1 cells express NMDAR1 receptor subunits, although expression of NMDAR1 in GnRH neurons, in vivo, is less clear (Gore, 2001; Urbanski et al., 1994, 1996; Mahesh et al., 1999). In contrast, results from electrophysiological recordings question the physiological relevance of these findings. For example, Spergel et al. (1999) clearly demonstrated that glutamate-induced currents in GnRH neurons are mainly mediated through AMPA receptors, not KA receptors, and to a lesser degree through NMDA receptors. Overall, our microarray results are in agreement with these electrophysiological data (Table 2 and Fig. 1). First, mRNA encoding KA2 was present in GT1-1 and GT1-7 cells, though not in Gn10 cells, whereas mRNAs encoding GluR5, GluR6 and