Journal of
Steroids & Hormonal Science
ISSN: 2157-7536

Su et al. J Steroids Hormon Sci 2011, S:2
DOI: 10.4172/2157-7536.S2-003

Review Article Open Access

Cell Models for the Study of Sex Steroid Hormone Neurobiology

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Abstract

To date many aspects of neurons and glia biology remain elusive, due in part to the cellular and molecular complexity of the brain. In recent decades, cell models from different brain areas have been established and proven invaluable toward understanding this complexity. In the field of steroid hormone neurobiology, an important question is: what is the profile of steroid hormone receptor expression in these specific cell lines? Currently, a clear summary of such receptor profiling is lacking. For this reason, we summarized in this review the expression of estrogen, progesterone, and androgen receptors in several widely used cell lines (glial and neuronal) derived from the forebrain and midbrain, based on our own data and that from the literature. Such information will aid in the selection of specific cell lines used to test hypotheses related to the biology of estrogens, progestins, and/or androgens.

Keywords: Androgen, Estrogen, Progesterone, GPR30, PGRMC1

Introduction

Sex steroid hormone receptors

The investigation of sex steroid hormone-brain interactions can be traced back to 1849, when A. A. Berthold transplanted testes into castrated roosters and observed the restoration of certain behavioral changes [1]. Sex steroid hormones (androgens, estrogens, and progestins) are known to mediate several biological effects, such as cell proliferation, differentiation, and homeostasis through their respective nuclear receptors. In addition to mediating transcriptional activities through their nuclear receptors, these hormones can mediate rapid activation through non-genomic signaling pathways [2].

The androgen receptor (AR) has two native ligands, testosterone and 5a-dihydrotesterone, which bind AR and activate transcription [3]. The AR is generally expressed as a single AR, and consists of N-terminal regulatory domain, DNA-binding domain, a small hinge region, and ligand binding domain [4]. The N-terminal regulatory domain mediates most of AR's transcriptional activity [5]. Two isoforms of AR (A, 87 kDa and B, 110 kDa) have been identified in peripheral tissue, in which AR-A has a truncated N-terminus compared to full length AR-B [6]. In addition, a splice variant of AR, known as AR45, has been found in heart and skeletal muscle, but the biological significance of this splice variant is unknown [7]. Further, membrane-associated binding sites of AR have been found in several cell types, such as endothelial cells, prostate cancer cells, and breast cancer cells [8-10]. Indicative of non-genomic signaling, membrane-associated AR (mAR) is sensitive to g-protein coupled receptor antagonists but not AR antagonists [9]. Functionally, mAR has been associated with increased intracellular calcium [9]. As of yet, mAR has not been cloned. Distribution of AR mRNA containing cells has been identified in the forebrain, midbrain, brain stem, and spinal cord [11,12].

The estrogen receptor (ER) is expressed as two proteins, ERα and ERβ, and produced from two different genes [13]. Binding of ER with estradiol, results in receptor dimerization and binding of ER to specific DNA sites in the gene promoter region, also known as the estrogen response element, to initiate transcription [14]. ERα generally has stronger transcriptional activation than ERβ whereas ERβ can attenuate ERα transcriptional activity [15-17]. In addition to ERα and ERβ, a g-protein coupled estrogen receptor, GPR30, has been shown to bind estrogen and mediate rapid non-genomic actions [18,19] through g-protein activation [20]. Interestingly, expression of GPR30 can be upregulated by progestins [19]. Both ERα and ERβ have been observed in neurons and glia [21]. ER expression has been observed throughout the brain [22-25]. Although ERα and ERβ normally have overlapping expression patterns, ERα is generally more widely expressed in brain regions involved in reproductive function, while ERβ is widely distributed in regions involved in anxiety, depression, learning, and memory [26]. Increasing evidence suggests that ERα activation is anxiogenic [27] (Morgan et al., Lund et al.), while ERβ activation is anxiolytic and antidepressive [27-32].

Similar to estrogen receptors, the progesterone receptor (PR) has two isoforms, PR-A and PR-B [13]. However, unlike the estrogen receptor, PR-A and PR-B are produced from the alternate use of two promoters from the same gene [13]. PR-A, a truncated form of PR, is lacking the first 164 amino acids in the N-terminal domain [13]. Functionally, PR-B is a stronger activator than PR-A, whereas PR-A functions as a ligand-dependent repressor of other steroid hormone transcriptional activity, including PR-B, ER, and AR [33-35]. In addition to PR-A and PR-B, a novel membrane-associated g-protein coupled PR (mPR) has been cloned [36]. However, the biological significance of mPR mediating non-genomic actions of progesterone is currently unclear. Lastly, the progesterone receptor membrane component 1(PGRMC1) is a member of the multi-protein binding complex regulated by progesterone, but its role in progesterone signaling is unknown [37-42]. This receptor has no homology similar to nuclear or membrane-associated steroid receptors [43]. Further, this receptor does not directly bind progesterone, but does activate P450 proteins involved in cholesterol synthesis and has been implicated in controlling membrane-associated signaling [44-46]. Distribution of PR has been localized to many brain regions, including the forebrain, midbrain, and brain stem [47-52].

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Received September 06, 2011; **Accepted** October 11, 2011; **Published** October 15, 2011

Citation: Su C, Rybalchenko N, Schreihofer DA, Singh M, Abbassi B, et al. (2011) Cell Models for the Study of Sex Steroid Hormone Neurobiology. J Steroids Hormon Sci S2:003. doi:10.4172/2157-7536.S2-003

Clonal cell lines

The field of steroid hormone neurobiology has benefited greatly from our growing knowledge of putative sex steroid hormone receptor sites and mapping of their distribution. Despite this progression, to date many aspects of the biology of neurons and glia remain elusive, mainly due to the cellular and molecular complexity of the brain. In addition, it remains technically challenging to perform *in vivo* studies on molecular mechanisms of hormonal regulation, receptor activation, intracellular signaling, and promoter/transcriptional regulation, with cells of different types and developmental origins. One way to reduce this complexity is to use primary neuronal and glial cultures. Nevertheless, non-transformed primary cultures may not always be ideal because of their short life span, and may represent a heterogeneous cell population. In order to overcome these issues, numerous labs have taken advantage of clonal cell lines, which are readily available, can go through multiple passages, and often represent a much more homogenous population of specific cell types with fewer uncontrolled variables than the *in vivo* situation.

From a historic perspective, the principles of brain cell culture began in the late 19th century when the German embryologist Wilhelm Roux successfully removed the neural plate from chicken embryos and maintained it in warm saline solution for several days [53]. The groundwork laid down by pioneers, such as Sydney Ringer and Ross Harrison, was advanced significantly in the 1940s and 1950s [54]. The first immortalized cell line was developed by W. Earle and colleagues from mouse connective tissue in 1943 to bypass the limitation of cell senescence and death [55]. In 1984, Cepko developed retroviral shuttle vectors utilizing SV40 T-antigen-mediated replication, thus allowing researchers to retrovirally infect primary mammalian cells with an immortalizing oncogene and selectively propagate them [56]. These technologies paved the way for a completely new approach to scientific investigation using clonal cell lines as an *in vitro* model to study neurobiology.

In recent decades, cell models from different brain areas have been established and proven invaluable toward understanding the cellular biology of specific hormone-sensitive cells. Some commonly used cell lines include the GnRH GT1-7 neurons from hypothalamus, cholinergic CNh neurons from cerebral cortex, dopaminergic N27 neurons from midbrain, as well as glial C6 cells from glioma. Such cell lines represent distinct neuron and glia types, which express a specific array of receptors, neurotransmitters and neuropeptides. They retain, at least in part, the major functional features of the cells from which they originated, and make detailed cellular and molecular studies possible. Although cell lines lack the complexity of an intact brain and do not exhibit the same integrated network of cellular interconnections and signaling, results from cell lines have been shown to largely recapitulate the *in vivo* environment when studying effects such as hormonal regulation, gene expression, and receptor activation/signaling.

However, a clear summary of sex steroid hormone receptor profiling in cell lines is lacking. For this reason, we summarized, based on our own data and that from the literature, the expression of estrogen, progesterone, and androgen receptors in several widely used cell lines (glial and neuronal) derived from the forebrain, midbrain, and glia.

Forebrain Cell Lines

The forebrain, a component of the central cholinergic system, is a region of the brain associated with higher order processing, such as learning, memory, perceptual awareness, attention, language, consciousness, and emotional behavior [57-60]. Important structures located within the forebrain include the thalamus, hypothalamus, limbic system, basal ganglia, and cerebral cortex [61]. Increased interest in the function of the forebrain has occurred with increased knowledge of Alzheimer's disease, a form of progressive dementia affecting memory, thinking, language, perception, and emotional behavior [62]. Decreased cholinergic markers in the cerebral cortex, correlating with cortical pathology and cognitive impairment, have been found in patients diagnosed with Alzheimer's disease [63,64]. Interestingly, steroid hormones have been shown to have a neuroprotective quality in models of Alzheimer's disease [65-69].

Cerebral cortical cell lines

With increased knowledge of Alzheimer's disease, cerebral cortical cell lines have been utilized more. The most commonly used cortical cholinergic cell lines are derived from humans or mice. However, knowledge about steroid hormone receptors is scarce.

The HCN cell line is derived from cortical tissue removed from a patient undergoing hemispherectomy for intractable seizures, and it is positive for glutamate, somatostatin, gamma aminobutyric acid (GABA), and cholecystokinin [70]. Studies have shown that the HCN line is responsive to estrogen, in which estrogen protected cells from glutamate-induced toxicity and oxidative stress-induced cell death [71,72]. Characterization of this cell line for steroid hormone receptors has not been reported. However, partial steroid hormone characterization has been completed in another human cell line. The IMR-32 cell line is a human neuroblastoma cell line, derived from an abdominal mass, which mimics large projections of the cerebral cortex [73]. This line has both cholinergic and adrenergic properties [74-77]. This cell line does not express estrogen receptor alpha (ERα) [78].

Two commonly used cerebral cortical lines derived from mice are the CNh and the NG108-15 cell lines. The cholinergic CNh cell line expresses glutamate receptors [79,80], but no studies have been reported on the effects of hormones or presence of steroid hormone receptors in this line. In contrast, the cholinergic NG108-15 hybridoma (mouse neuroblastoma x rat glioma C6) cell line [81] has been utilized in hormone assays studies. This cell expresses both ERα mRNA and ERβ, protein, but does not express androgen receptors (AR) [82-84].

Limbic region cell lines

The limbic region is involved in emotional behavior, metabolic processes, activation of the autonomic nervous system, learning, and memory [57]. Structures that make up the limbic region include the hippocampus, olfactory cortex, septum, hippocampus, and other nuclei [57]. Steroid hormone characterization of cell lines derived from these structures has been conducted in our laboratories and other laboratories.

Three widely used hypothalamic cell lines include the GT1-7, mHypoE-46, and the GNV. The GT1-7 cell line is the most studied hypothalamic cell line to date. This gonadotropin-releasing hormone (GnRH) line was derived from a mouse hypothalamic SV40 transformed tumor [85]. This cell line mimics *in vivo* rhythmic pulsatile GnRH secretion and forms networks in culture [85]. We have found mRNA for ERα, ERβ, and g-protein coupled estrogen receptor 1 (GPR30/GPER) (Table 1), along with protein expression for AR [86]. Both ERβ mRNA and protein, and AR protein have consistently been shown to be present in GT1-7 cell lines [87-92]. Further, GT1-7 has been shown to bind androgens [93]. Other steroid receptors present in GT1-7 cells are the progesterone receptor (PR) and the glucocorticoid receptor

(GR) [94,95]. However, the presence of ERα is unclear. Some studies report the presence of mRNA and protein for this receptor and binding of estrogen in GT1-7 cells [87-90,96], consistent with *in vivo* expression of ERα and ERβ receptors in GnRH neurons [97]. However, others have shown the absence of the ER receptor [94]. These differences in steroid hormone receptor profile may be partially mediated by the widely varied culturing conditions. Generally, DMEM or DMEM/Ham's F-12 (1:1) media is used with 10% fetal bovine serum (FBS) [87-90,94] (Table 1), although one laboratory did use 5% horse serum and 5% fetal calf serum (FCS) instead of FBS [96]. Wide variations in supplements were also observed, consisting of sodium pyruvate, sodium bicarbonate, glucose, Glutamax, or L-glutamine added to the culture media [87-89,94,96].

Fewer studies have been reported on steroid hormone receptor profile in the mHypoE-46 and GNV cell lines. The mouse-derived mHypoE-46 cell line consists of NPY-expressing neurons [98]. The profile of steroid hormone receptors in this line consists of AR, GR, ERα, and ERβ mRNA [95]. Less is known about the GNV conditionally immortalized GnRH cell derived from an adult rat [99]. Only ERα and ERβ receptors have been investigated and shown to be present at this time [95].

Two other GnRH cell lines have been used. The GN11 and NLT cell lines are derived from the same mouse olfactory tumor [100]. They both exhibit features of immature olfactory neurons, and have rhythmic pulsatile GnRH secretion [101]. However, the NLT cell line has a higher GnRH secretion than the GN11 line [100]. Our laboratory has found GPR30, ERα, and ERβ mRNA in both cell lines (Table 1). Other laboratories have also shown ERα and ERβ mRNA and binding of radiolabeled estrogen to GN11 cells [87,94]. Additionally, the GN11 cell line contains PR mRNA, including membrane-associated PR (mPR) [94].

A widely used cholinergic cell line is the SN56 line that releases luteinizing hormone-releasing hormone (LHRH) [102]. This cell line is derived from a mouse septal neuroblastoma [103]. Our laboratory has found that the SN56 cells contain mRNA for GPR30, ERα, ERβ, PR, mPR, progesterone receptor membrane component 1 (PGRMC1), and AR (Table 1). Other laboratories have also found ER mRNA and protein expression, along with PR mRNA [104-106].

Two commonly used hippocampal-derived cell lines are the HT22 and the HN33 cells [107,108]. Our laboratory found mRNA for ERα, ERβ, PGRMC1, and GPR30 and no mRNA PR, and AR in HT-22 cells. ERs in HT22 have been controversial. Several laboratories report the presence of ERα[106,109] and ERβ [110], while other laboratories report the absence of ERα [111-115] and ERβ [109,111,115,116]. Interestingly, we have found that culturing conditions alter mRNA levels for ERβ in HT22 cells (Table 1). Generally, DMEM media is used, but serum supplementation and antibiotic usage can vary (i.e. FBS or FCS with either penicillin/streptomycin or gentamycin) [106,109-116], indicating that culturing conditions may alter steroid hormone receptor expression. However, even less is reported in the literature about the steroid profile of the HN33 cell line. This cell line is a fusion of mouse hippocampal cells and N18TG2 neuroblastoma cells [108]. These cells display characteristics typical to hippocampal neurons, not neuroblastomas [117,118]. As of this date, our laboratory is the only one that has profiled the HN33 cells for steroid receptors. We have found GPR30, ERα, and ERβ steroid receptors (Table 1).

Midbrain Cell Lines

Major sources of catecholamines and catecholamine-releasing neurons are within the midbrain [119]. The midbrain is associated with motor control, cognition, emotion, and motivational control [120-123]. Structures located with the midbrain include the substantia nigra, ventral tegmental area, and red nucleus [120]. Several pathological conditions are associated with the midbrain, such as Parkinson's disease, schizophrenia, and Tourette's syndrome [120]. Further, steroid hormones can modulate catecholaminergic neurons in the midbrain [124-126].

Catecholamine cell lines

The SH-SY5Y catecholamine cell line is derived from a human neuroblastoma and considered genetically female [127,128]. This cell

Cell Line	PR (t)	PR (p)	mPR-α (t)	mPR-β (t)	PGRMC1(t)	ERα (t)	ERα (p)	ERβ (t)	ERβ (p)	GPR30 (t)	GPR30 (p)	AR (t)	AR (p)
GN11						++		++		++			
GT1-7						+		++		++			++
HN33						++	++	++	+	++			
HT22	-		-	-	+++	+++		+		+		-	
HT22*						++	+++	++	+	+			
NLT						++		++		++			
SN56	+		+	-	+++	++		+		+++		++	

Table 1: Steroid Hormone Receptor Profile for Forebrain Cell Lines.
+ = receptor level expression, t = mRNA transcript, p = protein expression
This data was obtained from our laboratory and all cell passage numbers were less than 20.
Culture condition for each cell type: GN-11 (DMEM + 10% fetal bovine serum (FBS) + 1% penicillin- streptomycin (PS), GT1-7 (DMEM + 10% FBS + 1% PS), HN33 (DMEM + 10% FBS + 1% PS), HT22 (DMEM +10% charcoal-stripped FBS + 20ug/ml gentamycin), HT22* (DMEM + 10% FBS + 1% PS), NLT (DMEM + 10% FBS + 1% PS), SN56 (DMEM + 10% FBS + 1% PS).

Cell Line	PR (t)	PR (p)	mPR-α (t)	mPR-β (t)	PGRMC1(t)	ERα (t)	ERα (p)	ERβ (t)	ERβ (p)	GPR30 (t)	GPR30 (p)	AR (t)	AR (p)
CAD	++		++	-	++		+						
N27	-					+	+	+				-	+
N2A						++	+	++	+	+++			
PC-12	++		+++	++	+++	-		+				-	
SH-SY5Y	-		-	-		-		-					

Table 2: Steroid Hormone Receptor Profile for Midbrain Cell Lines.

+ = receptor level expression , t = mRNA transcript, p = protein expression

This data was obtained from our laboratory and all cell passage numbers were less than 20, except PC-12. PC-12 cells were passaged between 80-90. Culture condition for each cell type: CAD (DMEM/F12 + 8% FBS + 1% PS), N27 (RPMI + 10% FBS + 1% PS), N2A (MEM + 10 % FBS + 1% PS), PC-12 (RPMI + 5% FBS + 10% heat inactivated horse serum + 1% PS), SH-SY5Y (DMEM/F12 + 10% FBS + 1% PS).

Cell Line	PR (t)	PR (p)	mPR-α (t)	mPR-β (t)	PGRMC1(t)	ERα (t)	ERα (p)	ERβ (t)	ERβ (p)	GPR30 (t)	GPR30 (p)	AR (t)	AR (p)
C6	-		++	++	+++	-		+				-	+
T98G	+		++	++		-		-					

Table 3: Steroid Hormone Receptor Profile for Glial Cell Lines.

+ = receptor level expression, t = mRNA transcript, p = protein expression

This data was obtained from our laboratory and all cell passage numbers were less than 20. Culture condition for each cell type: C6 (DMEM + 10% FBS + 1% Gentamicin), T98G (MEM + 10% FBS + 1% PS).

Cell Line	PR (t)	PR (p)	mPR-α (t)	mPR-β (t)	PGRMC1(t)	ERα (t)	ERα (p)	ERβ (t)	ERβ (p)	GPR30 (t)	GPR30 (p)	AR (t)	AR (p)	other
A172						-							+	
C6	-	-	+	+	+	-/+	-/+	-/+	-/+			-	+	
CAD	+		+	-	+		+							
Cath.a							-		+					responsive to steroid hormones
CNh														
GN11	+		+	+		+	+	+		+				
GNV						+		+						
GT1-7	+	+	+			-/+	+	+	+	+			+	
HCN														responsive to estrogen
HN33						+	+	+		+				
HT22	-		-	-	+	-/+	-/+	-/+	-/+	+		-/+		mERα, **Erβ splice variant**
IMR-32						-								
mHypoE-46							+		+				+	
N27	-					+	+	+				-	+	
N2A	+					+	+	+	+	+		+		**Erβ splice variant**
NG108-15						+			+			-		
NLT						+		+		+				
PC-12	+		+	+	+	-		+	+	+	+	-/+	+	
SH-SY5Y	-		-	-		-	+	-	+			-	-	delta 7ERα
SN56	+		+	-	+	+	+	-/+		+		+		mERα
T98G	+		+	+		-/+		-				+	+	
U373MG						+		-						

Table 4: Steroid Hormone Receptor Profile for Reported Cell Lines.

Bold font indicates data only reported from our laboratory, while underlined font indicates data from our laboratory that is consistent with previous reports. t = mRNA transcript, p = protein expression

line is dopaminergic, cholinergic, glutamatergic, and adenosinergic [128]. Our laboratory has been unable to find the presence of mRNA for ER or PR in this line (Table 2). Other laboratories have confirmed this reported absence of the AR [129]. However, protein expression of ERα and ERβ in SH-SY5Y cells has been shown in other laboratories [130,131]. Culturing conditions may explain this discrepancy in steroid hormone receptor expression. Our laboratory used DMEM/F12 media with 10% FBS, whereas ER-positive cells were cultured in RPMI media with 10% FBS and Glutamax supplementation [130,131]. Further, in ER-positive cells it is possible that the ERα is not functional, since the dominant negative form of ERα protein, delta7-ERα, is in higher proportion to full length ERα protein [131]. Loss of neuronal characteristics has been described with increased passages and this line is not recommended for use after twenty passages.

The rat mesencephalic-derived cell line, N27, is a catecholamine cell line [132]. This cell line produces dopamine, has tyrosine hydroxylase activity, and is positive for the dopamine transporter [132]. Our laboratory has found mRNA for ERα and ERβ but not AR or PR (Table 2). However, AR protein expression along with decreased cell viability and increased activation of the PKCδ pathway in response to androgens was observed, supporting the hypothesis that a putative membrane associated androgen receptor might mediate this responsiveness to androgens [86].

The PC-12 cell line is derived from a pheochromocytoma of the rat adrenal medulla [133], and is catecholaminergic with catecholamine synthesizing enzymes and monamine oxidase activity [134,135]. This cell line switches from a dividing neuroblastoma phenotype to a non-dividing, neurite-bearing phenotype resembling mature sympathetic neurons when exposed to nerve growth factor (NGF) [133]. Our laboratory has found mRNA for PR, mPR, PGRMC1, ERβ, and no mRNA for ERα and AR (Table 2). Other laboratories have found ERβ and GPR30 protein expression, but no ERα in PC-12 cells [136,137]. However, mRNA and protein expression for AR have been reported [138,139]. This difference in AR expression could be due to Glutamax supplementation, since the media and serum used are similar to what is used in our laboratory [138,139] (Table 2).

The Cath.a cells are a catecholamine (dopamine and norepinephrine) synthesizing line that has a neuronal phenotype resembling locus coeruleus neurons [140]. These cells are derived from a brain tumor in a transgenic mouse with a SV40 T antigen under the transcriptional control of the rat tyrosine hydroxylase promoter [140]. Cath.a cells are responsive to estrogen, testosterone, and progesterone, as evidenced by altered CREB1 promoter activity [141]. Further, these cells express ERβ protein but not ERα [142].

The catecholaminergic CAD cell line is a variant of the Cath.a cells [140]. The CAD cells differ from Cath.a by loss of the immortalizing oncogene [143]. These cells exhibit characteristics similar to primary neurons [143]. Our laboratory has found the presence of mRNA for PR, mPR, and PGRMC1 (Table 2). However, recent reports show no PR protein expression in CAD cells [144].

Neural Crest Cell Line

The undifferentiated mouse neuroblastoma cell line N2A, also known as neuro2A, is a neural crest derived cell line [145]. This cell line expresses TH and can be differentiated into catecholaminergic cells [146,147]. Our laboratory has found mRNA for GPR30, ERα, and ERβ (Table 2). This data is consistent with results in the literature showing that estrogen binds to receptors in N2A and the presence of mRNA and protein expression for both ER subtypes [115,148]. In addition, mRNA for AR and PR has been reported [148].

Glial Cell Lines

In the central nervous system, approximately 90% of the cells are glia [149]. Glia have been shown to have several functions, including serving as a transportation link between the bloodstream and neurons, uptake of neurotransmitters, synthesis and release of neurotrophic factors, immune regulation, and modulation of synaptic activity [150-154]. Alzheimer's disease and Parkinson's disease have been associated with glial pathology [155,156]. Further, steroid hormones interactions with glia have been reported [157,158].

A commonly used glial cell line is the C6 rat glioma astrocytoma [159]. Our laboratory has found the presence of mRNA for mPR, PGRMC1, and ERβ, but not PR, AR, and ERα (Table 3). We have previously shown the presence of AR protein expression, indicating a putative membrane associated AR [160]. The presence of ER in C6 cells has been controversial. Some reports failed to detect ERs [161], whereas others note the presence of ERα and the absence of ERβ [162], or expression of both ERs [163]. These differences in steroid hormone receptor profile may partially be mediated by the widely varied culturing conditions, in which there is no consistency in media preference for C6 (i.e. DMEM, DMEM/F12, or RPMI) (Table 3).

Other glial cell lines include the human glioblastoma derived A172, T98G, and U373MG. Less information about the steroid receptor profiles of these lines are available. The A172 glial line is ER negative [164], but does express AR protein [165]. The T98G cells have mRNA for ERα and mPR, but not ERβ, and protein expression for AR [162]. Further, our laboratory has found the presence of mRNA for PR but not ER in T98G glial cells (Table 3). Lastly, mRNA for ERα, not ERβ, has been reported in the U373MG glial line [162].

Conclusion

While our knowledge from established central nervous system cell lines grows, concerns arise, since varied or even conflicting conclusions can be drawn from the same model. Varied observations might reflect differences in cell culture phenotype in different investigators' laboratories, which in turn may be due to culturing conditions, passage numbers, and so on. The formulation of cell culture media can also impact steroid hormone function [113,166]. For example, RPMI 1640 media contains glutathione but MEM, F12, and DMEM do not (see Invitrogen.com). The presence of glutathione in cell culture media has been shown to increase the potency of estrogens several hundred fold [113,166]. Therefore leading to an important question for investigators in the field of steroid hormone neurobiology: what is the profile of the sex steroid hormone receptor expression in the specific cell line being used? A thorough screening for receptors present in cells utilized by a specific lab is critical before choosing a model; yet, current literature lacks a clear summary, or at a minimum, a good guide of such profiling. For this reason, the present review is a progress report of these efforts from our own laboratory as well as others. This review provides an important insight of the utilization of clonal cell lines as our tools to investigate the mechanisms of steroid hormones in brain (Table 4).

Acknowledgements

We would like to thank Dr. David Schubert for the kind gift of HT-22 cells. This work was supported by the following grants: NIH F32 NS061417, AHA BGIA4180116, and UNTHSC Seed Grant to R.L.C. and NIH AG022550, AG027956, and an IIRG from Alzheimer's Association to M.S.

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This article was originally published in a special issue, **Androgen Receptors** handled by Editor(s). Dr. Rebecca L Cunningham, University of North Texas Health Science Center, USA; Dr. Ze-hua Liu, Osaka City University, Japan