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Ovarian steroids increase glutamatergic related gene expression in serotonin neurons of macaques ☆

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ABSTRACT

Dendritic spines are the elementary structural units of neuronal plasticity and their proliferation and stabilization involve components of glutamate neurotransmission. In a model of hormone replacement therapy (HT), we sought the effect of estradiol (E) and progesterone (P) on gene expression related to glutamate neurotransmission in a laser captured preparation enriched for serotonin neurons from rhesus macaques. Microarray analysis was conducted (n = 2 animals/treatment) and then confirmed for pivotal genes with qRT-PCR on additional laser captured material (n = 3 animals/treatment). Ovariectomized rhesus macaques were treated with either placebo, E or E + P via Silastic implants for 1 month prior to euthanasia. The midbrain was obtained, sectioned and immunostained for TPH. TPH-positive neurons were laser captured using an Arcturus Laser Dissection Microscope (Pixel II). RNA from laser captured serotonin neurons (n = 2 animals/treatment) was hybridized to Rhesus Affymetrix GeneChips for screening purposes. There was a 2-fold or greater change in the expression of 28 probe sets related to glutamate processes in E and E + P treated animals. Quantitative (q) RT-PCR was conducted for 11 genes with a custom Taqman PCR array containing monkey specific primers and analyzed with ANOVA followed by Bonferroni's test. The log of the relative expression values indicated that in general, the responses to E and E + P were similar. Comparison of the relative expression or log relative expression in Ovx-controls to combined E and E + P treated groups with t-tests showed a significant increase in AMPA1 (GRIA1), AMPA2 (GRIA2), AMPA4 (GRIA4), NMDA2a (GRIN2A), metabotrophic glutamate receptor (GRM1), glutamine synthetase (GLUL), glutamate dehydrogenase (GLUD), glutamate cysteine ligase modifier subunit (GCLM), the glutamate transporter 2 (SLC1A2) and the glutamate transporter 3 (SLC1A3) with steroid treatment. There was no effect of steroid treatment on gene expression of the glutamate cysteine ligase catalytic subunit (GCLC). These data suggest that ovarian steroids target gene expression of ionotrophic and metabotrophic glutamate receptors in serotonin neurons. These receptors are present on dendritic spines and are necessary for spine maturation. The mRNAs coding for glutamate-related enzymes and transporters are likely derived from astrocytes or glutamate-containing terminals. Their induction by ovarian steroids indicates a complex upregulation of multiple components in the glutamate cycle and antioxidation, in addition to spine proliferation.

Introduction

The serotonin system modulates a wide range of neural outcomes from emotion to intellect to metabolism and it is a target of pharmacotherapies, steroid hormones, cytokines, neuropeptides and trophic factors, all of which impact the generation and efficacy of serotonin neurotransmission. We recently reported that ovarian steroids regulate gene and protein expression in laser captured serotonin neurons in a manner that would increase cellular resilience and decrease apoptosis (Bethea and Reddy, 2008; Tokuyama et al., 2008). In addition, ovarian steroid administration decreased DNA fragmentation in serotonin neurons (Bethea et al., 2009; Lima and Bethea, 2009).

It has been suggested that antidepressants and other pharmacotherapies may act by promoting neuronal plasticity (Manji et al., 2003). The underlying structural element of neuronal plasticity in the adult nervous system is the dendritic spine (Ethell and Pasquale, 2005). In addition, dendritic spines are the morphological basis for excitatory neurotransmission (Butler et al., 1998; McKinney et al., 1999). A significant body of literature has demonstrated that estrogen (E) increases dendritic spines in the hippocampus and cortex (Cooke and Woolley, 2005; Hao et al., 2003, 2006; Murphy et al., 1998). We

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postulated that steroid-induced dendritic spine proliferation on serotonin neurons would have a profound effect on serotonergic function and neurotransmission.

The issue of steroid supported dendritic spine proliferation on serotonin neurons may be important for menopausal women grappling with issues surrounding hormone therapy (HT). Women experience ovarian failure and loss of ovarian steroid production around 50 years of age. Thus, with extended life spans, a woman may live 35–40 years without ovarian steroids. If dendritic spines on serotonin neurons shrink or atrophy due to lack of steroid supported gene expression, then geriatric depression, anxiety, fretfulness, decreased coping skills and increased vulnerability to stress can be predicted outcomes.

We recently reported that ovarian steroids increase gene expression in laser captured serotonin neurons for the effector GTPase proteins called CDC42, Rac 1 and Rho A in monkeys (Bethea and Reddy, 2010). These small molecules activate cascades that lead to filopodia extension, spine head enlargement and spine shortening, all of which are necessary for the production of a mature dendritic spine. Hence, if ovarian steroids enhance serotonin neuronal plasticity by increasing spine proliferation on serotonin dendrites, this would facilitate excitatory input to the serotonin neurons and in turn, increase serotonin neural activity.

A critical component of spine maturation and stability is the excitatory ionotrophic glutamate receptors, AMPA (α-amino-3-hydroxy-5-methyl-isoxazoleproprionate) and NMDA (*N* methyl-D-aspartate type). The synthesis, targeting, insertion and clustering of these receptors on spines are highly regulated in time scales ranging from minutes to months. We hypothesized that ovarian steroid administration would increase expression of AMPA and NMDA receptors in laser captured serotonin neurons as a reflection of increased spine proliferation. In addition, estradiol induces spines and the ionotrophic glutamate receptors by enhancing glutamate release independent of transcription (Schwarz et al., 2008). However, upregulation of NMDA receptors also follows NMDA antagonism or blocking of synaptic activity by tetrodotoxin (Bolton et al., 2000). Therefore, we also examined other components of glutamatergic organization since our laser captured serotonin preparation is only enriched for serotonin neurons and likely included glia and presynaptic elements.

Our model involves ovariectomy of adult rhesus macaques for 5–8 months, followed by subcutaneous delivery of estrogen (E) or estrogen plus progesterone (EP) for one month. Therefore, we can only examine one time point, which probably reflects a significant level of maturity and stabilization. We examined the effect of E, with and without supplemental P, on multiple genes related to glutamatergic function in a laser capture preparation enriched for serotonin neurons of rhesus monkeys using the Rhesus Affymetrix cDNA array and quantitative (q) RT-PCR. Eleven pivotal gene changes predicted by the microarray were examined by qRT-PCR.

Results

Expression changes related to glutamate receptors and enzymes in laser captured serotonin neurons

The entire data set of the 6 Affymetrix microarray chips has been submitted to the Gene Expression Omnibus public database (www.ncbi.nlm.nih.gov/geo/info/linking.html) and assigned the GEO accession number GSE16169. Table 2 contains the average signal intensities for the probe sets corresponding to genes related to glutamate receptors and enzymes in laser captured serotonin neuron preparations from duplicate animals/microarray chips in each group. The first 11 genes were further examined with qRT-PCR. The last 6 genes were interesting, but not pursued. Note that GAD1, GAD2, PAG and VGLUT2 increased, whereas SLC1A4 and GRIK2 (kainate receptor), decreased with steroid treatment. Hence, up and down regulation was observed on the microarray, but only increases were verified with qRT-PCR.

The relative expression values from the Taqman qRT-PCR array for each gene are shown in Table 3. Each animal was assayed in triplicate and a mean for the animal was obtained. Then the overall mean of the animals was obtained and subjected to statistical analysis. With this analysis, 6 of the 11 genes showed an increase in expression in the E and/or E + P treated groups. The expression of two genes, GLUS and GLUD2, was reduced to control levels when P was added to the E regimen. AMPA1, GRM1, GCLC, and the 2 transporters did not exhibit statistical significant changes. However, the variance was significantly different between the groups in this analysis by F test. Therefore, a log transformation was performed on the data.

Glutamate receptor expression changes

The log transformations of the relative expression of the receptor genes are illustrated in Fig. 1. There was a significant difference between groups in the expression of the AMPA1 subunit (ANOVA, $p = 0.02$). E treatment increased AMPA1 expression relative to Ovx-controls, but was not different from E + P treatment (Bonferroni, $p < 0.05$). There was a significant difference between groups in the expression of the AMPA2 subunit (ANOVA, $p = 0.0025$), the AMPA4 subunit (ANOVA, $p = 0.0001$) and the NMDA2a receptor (ANOVA, $p = 0.002$). Relative to the Ovx-control group, E and E + P treatment increased expression of AMPA2 (Bonferroni, $p < 0.05$, both treatments), AMPA4 (Bonferroni, $p < 0.05$, both treatments) and expression of the NMDA2A receptor (Bonferroni, $p < 0.05$, both treatments). There was no difference between the E- and E + P-treated groups. There was a near significant difference between groups in the expression of the metabotrophic glutamate receptor or GRM1 (ANOVA, $p = 0.08$). However, there was a significant increase in GRM1 with E only treatment compared to Ovx-controls as indicated with an unpaired, 2-tail t-test ($p = 0.006$).

Table 1
Available information about ABI custom Taqman qPCR assays.

	Gene symbol	Assay	Context sequence	NCBI gene reference
Glutamate receptor, ionotropic, AMPA 1	GRIA1	Rh02829827_m1	CCTTGCAAATCTGGGCTTCATGGAC	XM_001111076.1
Glutamate receptor, ionotropic, AMPA 2	GRIA2	Rh02790124_m1	AGGTGATTCCAAGGAAAAGACCAGT	XM_001095129.1, AY723727.1
Glutamate receptor, ionotropic, AMPA 4	GRIA4	Rh02829840_m1	CTCAAACAGGTTCGAATTCAAGGGC	XM_001101031.1
Similar to glutamate [NMDA] receptor subunit epsilon 1 pre	LOC715345	Rh03986722_m1	ATGATCATGGCTGACAAGGATCCGA	XM_001105525.1
Glutamate receptor, metabotropic 1	GRM1	Rh01068380_m1	TCTCACTCATTGGAAGTGATGGATG	XM_001085942.1
Glutamate-ammonia ligase (glutamine synthetase)	GLUL	Rh02928658_m1	CCTGCCCAGTGGGAATTTCAAATTG	XM_001114827.1
Glutamate dehydrogenase	GLUD	Rh03986729_m1	AAGCCCCGAGGCTTCTGCATTTGCT	XR_009675.1; NM_012084.1
Glutamate-cysteine ligase, modifier subunit	GCLM	Rh00978072_m1	GCTGTATCAGTGGGCACAGGTAAAA	XM_001103381.2; NM_002061
Glutamate-cysteine ligase, catalytic subunit	GCLC	Rh02838909_m1	CATCATCAATGGGAAGGAAGGCGTG	XM_001108857.1
Solute carrier family 1 (glial high affinity glutamate transport)	SLC1A2	Rh02847723_m1	GAGTGCAAGGTAACTCTGGCAGCCA	XM_001115008.1, AY369953.1
Solute carrier family 1 (glial high affinity glutamate transport)	SLC1A2	Rh00904820_m1	CCTGGACTTGATCAGGAACATGTTC	XM_001094417.1